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(54) Title: METHODS FOR THE IDENTIFICATION OF AGENTS FOR THE TREATMENT OF SEIZURES, NEUROLOGICAL DISEASES, ENDOCRINOPATHIES AND HORMONAL DISEASES

(57) Abstract: The present invention is drawn to methods of characterization of the properties and functions of SV2 proteins. The invention further includes methods of identifying compounds or agents which modulate the activity of SV2 proteins. Included in these methods is the identification of compounds or agents which modulate the binding of levetiracetam to SV2 proteins, including SV2A. Additionally, the present invention provides biotinylated ligands as a tool to screen chemical libraries and characterize the SV2 proteins. Further, the present invention provides a method of solubilizing and purifying functionally active membrane associated proteins, such as SV2.

METHODS FOR THE IDENTIFICATION OF AGENTS FOR THE TREATMENT OF SEIZURES, NEUROLOGICAL DISEASES, ENDOCRINOPATHIES AND HORMONAL DISEASES

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RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application 60/506,764, filed September 30, 2003, and U.S. Provisional Application 60/430,372, filed December 3, 2002, which are herein incorporated by reference in their entirety.

FIELD OF THE INVENTION

The present invention is generally drawn to the field of drug discovery in neurological disorders, endocrinopathies and hormonal diseases.

BACKGROUND OF THE INVENTION

Neurological disorders afflict a substantial number of individuals and present an increasing economic challenge to health care systems since little is known regarding their causes, their diagnosis is often subjective, and many lack effective treatment. In general, brain activity is ultimately determined by the capacity of neurons to communicate at synapses. Specific neurotransmitter chemicals are packaged in presynaptic neurons into synaptic vesicles which fuse with the presynaptic membrane to release *quanta* of the neurotransmitter chemical that traverse the synaptic cleft to activate the corresponding receptor type resident in the post-synaptic membrane. Among these receptor types are the neuronal glutamate receptors (GluR's), γ-aminobutyric acid receptors (GABAR's), nicotinic acetylcholine receptors, serotonin receptors, dopamine receptors, and the like. Many neurological disorders are a result of improper conduction of electrical currents through synapses in various brain tissues. In epilepsy errant currents, hypothesized to be associated with improper function of synapses, cause various levels of seizures. Likewise, in several psychiatric diseases, movement disorders and neurodegenerative diseases the conduction currents become aberrant, disorganized or reduced, thereby causing the disease condition.

Accordingly, defects in synaptic vesicle functions will have an adverse effect upon neurotransmission in general and control of neurotransmitter release in particular.

Seizures, including epileptic seizures, result from a focal or generalized disturbance of cortical function, which may be due to various cerebral or systemic disorders, including, for example, cerebral edema, cerebral hypoxia, cerebral trauma, central nervous system (CNS) infections, congenital or developmental brain defects, expanding brain lesions, hyperpyrexia, metabolic disturbances and the use of convulsive or toxic drugs. It is only when seizures recur at sporadic intervals and over the course of years (or indefinitely) that epilepsy is diagnosed.

Epilepsy is classified etiologically as symptomatic or idiopathic with seizure manifestations that fall into three general categories: 1) generalized tonic-clonic, 2) absence or petiti mal, and 3) complex partial. Symptomatic classification indicates that a probable cause exists and a specific course of therapy to eliminate that cause may be tried, whereas idiopathic indicates that no obvious cause can be found and may be linked to unexplained genetic factors. Of the seizure categories, most persons have only one type of seizure, while about 30% have two or more types.

The risk of developing epilepsy is 1% from birth to age 20 yr. and 3% at age 75 yr. Idiopathic epilepsy generally begins between ages 2 and 14. Seizures before age 2 are usually caused by developmental defects, birth injuries, or a metabolic disease. Those beginning after age 25 may be secondary to cerebral trauma, tumors, or cerebrovascular disease, but 50% are of unknown etiology.

Due to the many interrelationships that exist between the nervous and endocrine systems, defects in synaptic vesicle function can also impact on endocrinological function. For instance, at least two glands secrete their hormones only in response to appropriate neurotransmitter release - the adrenal medulla and the posterior pituitary gland. Upon secretion, hormones are transported in the blood to cause physiologic actions at distant target tissues in the body. Obviously, endocrinopathies involving either hyper- or hyposecretion of hormones have pathological consequences. Exemplary of these consequences are giantism and dwarfism, due to hyper- or hyposecretion of growth hormone, respectfully.

Levetiracetam

Levetiracetam (LEV; ucb L059; (*S*)-α-ethyl-oxo-pyrrolidine acetamide),the (*S*)-enantiomer of the ethyl analog of piracetam, was synthesized during a follow-up chemical program aimed at identifying a second-generation nootropic drug. *In vivo* results have demonstrated an unexpected potent ability of LEV to suppress seizures in the audiogenic-susceptible mouse, whereas piracetam was only weakly active. Although LEV is a molecule unrelated to established antiepileptic drugs (Margineanu *et al.*, in Antiepileptic Drugs: 5th Edition. pp. 419-427. Lippincott, Philadelphia (2002)), extensive clinical trials have proven that adjunctive therapy with LEV (KEPPRA, UCB, S.A., Braine-l'Allend, Belgium) is both effective and well tolerated in controlling refractory partial seizures in adults.

Binding assays with LEV, performed on crude rat brain membranes, reveal the existence of a reversible, saturable and stereoselective specific binding site. Results obtained in rat hippocampal membranes suggest that LEV labels a single class of binding sites with modest affinity and with a high binding capacity. This binding site is identified as the Levetiracetam Binding Site (LBS). Similar results have been obtained in other brain regions (cortex, cerebellum and striatum). Ucb L060, the (R)-enantiomer of levetiracetam, displays about 1000 times less affinity for these sites. The binding of LEV appears to be confined to membranes in the central nervous system since radiolabel studies could detect no specific binding in a range of peripheral tissues including heart, kidneys, spleen, pancreas, adrenals, lungs and liver. However, this could be due to a low density of LBS in these tissues compared to the central nervous system and indeed specific binding does occur in PC12 cells, a peripherally derived adrenal cell line.

The most commonly used antiepileptic drugs carbamazepine, phenytoin, valproate, felbamate, gabapentin, tiagabine, vigabatrin, zonisamide, phenobarbital and clonazepam, as well as the convulsant agent t-butylbicyclophosphorothionate (TBPS), picrotoxin and bicuculline do not displace LEV binding (Gillard *et al.* Eor. J. Pharmacol. 478:1-9. (2003))). However, ethosuximide, pentobarbital, pentylenetetrazole and bemegride competed with LEV with pKi values comparable to active drug concentrations observed *in vivo*. Structurally related compounds, including piracetam and aniracetam, also displaced LEV binding. The levetiracetam analogues were also tested for their anticonvulsant activity in the audiogenic mouse model of epilepsy. A very good correlation (r^2=0.84) was observed between the

affinity and the anticonvulsant activity (Noyer *et al.*, Euro. J. Pharmacol. 286:137-146. (1995)). This high degree of correlation is strong support for a causative relationship between LBS binding and anticonvulsant activity of this class of compounds. Accordingly, binding of levetiracetam analogues to LBS is expected to result in modification of the function of the protein component(s) of the LBS in brain, leading to the desired therapeutic outcome of anticonvulsant activity.

The Synaptic Vesicle Protein 2 Family

The Synaptic Vesicle Protein 2 (SV2) family of synaptic vesicle proteins was first identified with a monoclonal antibody prepared against cholinergic vesicles from the electric organ of the marine ray *D. ommata* (Buckley *et al.*, J. Cell Biol. 100:1284-1294. (1985)). Cloning of the individual family members labeled by the antibody resulted in the identification of three different isoforms, SV2A (Bajjalieh *et al.*, Science. 257:1271-1273. (1992)), SV2B (Feany *et al.*, Cell. 70(5):861-867. 1992) and SV2C (Janz and Sudhof, Neuroscience 94(4): 1279-1290. (1999)), all of which react with the original antibody. The overall homology between the three rat isoforms is approximately 60%, with SV2A and SV2C being more similar to each other than SV2B (Janz and Sudhof, Neuroscience 94(4): 1279-1290. (1999)).

The SV2 proteins are integral membrane proteins and have significant but low-level homology (20-30%) to the twelve transmembrane family of bacterial and fungal transporter proteins that transport sugar, citrate, and xenobiotics (Bajjalieh *et al.*, Science. 257:1271-1273. (1992)). As putative members of the 12 TM superfamily, SV2 proteins display several unique features. They have relatively short free N- and C- termini and short loops connecting the Tm segments. Two notable exceptions, however, are the long cytoplasmic loop between transmembrane regions 6 and 7 and the intravesicular loop between transmembrane regions 7 and 8 (which contains 3 N-glycosylation sites). No close homologs of the SV2 proteins have yet been discovered in yeast or invertebrates, although a distantly related synaptic vesicle protein known as SVOP does have homologs in *Drosophila* and *C. elegans* (Janz *et al.*, J. Neurosci. 18(22):9269-9281. (1998)).

As a family, SV2 proteins are widely distributed in the brain and in endocrine cells. The three isoforms overlap significantly in their distribution, and can be found co-expressed

in the same neuron, and even on the same synaptic vesicle. One isoform or another of the SV2 proteins seems to be present on all synaptic vesicles, and they are probably not limited to neurons that contain any specific neurotransmitters, although one study reports that cholinergic vesicles may not contain SV2 (Blumberg *et al.*, J. Neurochem. 58(3):801-810 (1992)). SV2 proteins are therefore one of the most common proteins of synaptic vesicles, and have been implicated in the control of calcium-mediated exocytosis of synaptic vesicles. SV2 proteins have also been shown to be expressed in endocrine cells and, along with the additional synaptic vesicle membrane integral proteins p38 and p65, has been demonstrated to be present in endocrine dense core granule membranes (Lowe *et al.*, J. Cell. Biol. 106(1):51-59 (1988). SV2A, the most common SV2 isoform, is expressed ubiquitously throughout the brain, and is present as well in secretory granules of endocrine cells. SV2B, while broadly distributed in the brain, is undetected in several brain structures, including the dentate gyrus of the hippocampus, the globus pallidus, reticular nuclei of the thalamus, and the reticular part of the substantia nigra (Bajjalieh *et al.*, 1994). By contrast, SV2C has quite a limited distribution and is found primarily the phylogenetically old regions such as the pallidum, the substantia nigra, the midbrain, the brainstem and the olfactory bulb. It is undetectable in the cerebral cortex and the hippocampus, and found at low levels in the cerebellar cortex (Janz and Sudhof, Neuroscience 94(4): 1279-1290. (1999)).

In addition to the SV2 protein, the synapse contains other unique regulatory proteins such as synapsin, synaptotagmin and CAPS, which may mediate vesicle fusion or budding. SV2A may be a Ca^{2+} regulatory protein essential for the formation of pre-fusion complexes called SNARE complexes (Xu *et al.* Cell 99(7):713-722 (1999)), which include the synaptic vesicle-associated VAMP/synaptobrevin and the plasma membrane proteins syntaxin and SNAP-25. Upon Ca^{2+} accumulation in the synapse the binding of synaptotagmin to SV2A is inhibited and the dimerization of two synaptotagmin Ca^{2+} binding domains is stimulated (Bajjalieh, Curr. Opin. Neurobiol. 9(3):321-328. (1999)). This dimerization may play a role in organizing the SNARE complex and promoting vesicle fusion, as at low Ca^{2+} concentrations, SV2A remains bound to synaptotagmin and fusion will not occur.

The affinity of SV2A for synaptotagmin is regulated by the phosphorylation of the amino terminus of SV2 (Pyle *et al.*, J. Biol. Chem. 275(22):17195-17200. (2000)). The possibility that SV2 proteins play a role in either Ca^{2+} transport, or regulation in the synaptic

vesicle has been supported by studies of SV2A and SV2B knockout animals (Janz *et al.*, Neuron 24:1003-1016. (1999)). An alternative hypothesis is that the SV2 proteins, while derived from transport proteins, now serve a different function in the vesicle, whether a structural role or a role in regulation of vesicle fusion or recycling and the exocytotic release of their contents (Janz and Sudhof, Neuroscience 94(4): 1279-1290. (1999)).

There have been two reports of SV2 protein knockout mice: one that examines only SV2A knockouts (Crowder *et al.*, Proc. Nat. Acad. Sci. USA 96(26):15268-15273. (1999)) and the other which looks at both SV2A and SV2B knockout animals, as well as the SV2A/SV2B double knockout (Janz *et al.*, Neuron 24:1003-1016. (1999)).

Animals homozygous for SV2A gene disruption appear normal at birth, but fail to grow, experience severe seizures, and die within the first few weeks postnatal. SV2A homozygous knockout mice experience seizures that are longer lasting, stronger, and more debilitating than any other mouse strain (Janz *et al.*, Neuron 24:1003-1016. (1999)). Despite the appearance of postnatal seizures, all SV2A knockout animals have completely normal gross brain morphology, including normal levels of the tested synaptic proteins. Furthermore, the hippocampal neuronal cultures from both SV2A and SV2A/SV2B double knockout mice formed synapses that were ultrastructurally normal, and had unchanged size, number and location of synaptic vesicles (Janz *et al.*, Neuron 24:1003-1016. (1999); Crowder *et al.*, Proc. Nat. Acad. Sci. USA 96(26):15268-15273. (1999)). Unlike the frequently observed seizures caused by structural and developmental abnormalities easily detected in many other type of knockouts, the SV2A knockout mice show a strong seizure phenotype with no associated macro or micro scale abnormalities of the brain or synapse. As another marker of brain function, studies of synaptic transmission in primary neuronal cultures from SV2A, SV2B, and SV2A/SV2B knockout mice indicate that the sizes and frequencies of sIPSCs and of spontaneous excitatory postsynaptic currents (sEPSCs), are normal. Electrical stimulation induced robust EPSCs and IPSCs in the cultured neurons from all genotypes.

In contrast to SV2A, SV2B knockout mice reveal no overt pathology (Janz *et al.*, 1999). It is suggested that one possible reason for this lack of consequence of loss of SV2B is that can be functionally replaced by SV2A, which appears to be co-expressed everywhere SV2B is normally expressed.

While the function of SV2A and other family members still remains unknown, one

hypothesis is that this transporter homologue is a functional transporter for some common synaptic vesicle molecule. More specifically, there is evidence linking SV2A to the regulation of calcium-mediated vesicle exocytosis, and as a result, it is thought that it may be a Ca^{2+} transporter. SV2A and other family members may also have roles in the function of synaptic vesicles. Such roles may include modulating aspects of their formation, loading with neurotransmitter, fusion with the plasma membrane, re-cycling, and interactions with other proteins and cellular compartments and organelles. For instance it has been shown that SV2 proteins can interact with the synaptic vesicle protein synaptotagmin and the extracellular matrix protein laminin-1 (Carlson, Perspect. Dev. Neurobiol. 3(4):373-386 (1996)). The SV2 proteins may play important roles in regulating cytoplasmic or organellar calcium levels at the presynaptic terminal, and may also interact with N-type calcium channels on the plasma membrane, either directly or indirectly.

SUMMARY OF THE INVENTION

The present inventors have discovered that SV2A is the binding site for the anti-seizure drug LEV and its analogs. The high degree of correlation between relative binding affinities of a series of levetiracetam analogues and their anti-convulsant potencies in certain animal models of epilepsy provides strong evidence that binding of these analogues to SV2 proteins modifies their function to provide anticonvulsant effects.

In a preferred embodiment, the invention includes a method of treating a neurological disorder associated with synaptic vesicle function, endocrinopathy or hormonal diseases, comprising administering a compound or agent that modulates a function or activity of an SV2 protein.

In another preferred embodiment, the invention includes a method of discovering or modeling an interaction between an SV2 protein and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site. The method comprises contacting the SV2 protein with the compound or agent measuring and analyzing the interaction of the SV2 protein with the compound or agent.

In another preferred embodiment, the invention includes a method of identifying a

levetiracetam binding site within an SV2 protein. The method comprises contacting a SV2 protein or fragment thereof with a compound or agent selected from the group consisting of levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site and determining the binding of the compound or agent with the SV2 protein or fragment thereof.

In another preferred embodiment, the invention includes a method of assaying the interaction between SV2 protein and a second protein. The method comprises expressing SV2 protein and the protein of interest in a cell. The method further comprises exposing the cell to a compound or agent which binds to the levetiracetam binding site and determining the interaction between the SV2 protein and the protein of interest.

In another preferred embodiment, the invention includes a method of identifying a compound or agent that modulates a neurological disorder associated with synaptic function, endocrinopathy or hormonal disease. The method comprises exposing a SV2 protein to the compound or agent and determining whether the compound or agent modulates an activity of the SV2 protein.

In another preferred embodiment, the invention includes a method of identifying a cellular response to a compound or agent selected from the group consisting of levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site. The method comprises exposing cells expressing an SV2 protein to the compound or agent and analyzing a change in the expression of a nucleic acid or protein in the exposed cell. The nucleic acid could be RNA, and the expression of the RNA may be analyzed by hybridization, such as hybridization on a microarray.

In another preferred embodiment, the invention includes an isolated nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO: 5 or the complement thereof.

In another preferred embodiment, the invention includes a method of identifying a binding partner for a SV2 protein. The method comprises exposing a SV2 protein or fragment to a potential binding partner and incubating the protein or fragment and potential binding partner with (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide. The method further comprises determining if the binding of (2S)-2-[4-(3-azidophenyl)-2-

oxopyrrolidin-1-yl]butanamide to the protein is inhibited by the potential binding partner, thereby identifying binding partner for the protein.

In still another preferred embodiment, the invention includes a method of identifying an agent useful for the treatment of a neurological or endocrinological disorder. The method comprises exposing a SV2 protein or fragment to the agent and levetiracetam or an analog or derivative thereof. The method further comprises determining if the binding of levetiracetam or an analog or derivative thereof to the protein is modulated by the agent, thereby identifying an agent useful for the treatment of a neurological or endocrinological disorder.

In yet another preferred embodiment, the invention includes a method of identifying an agent useful for the treatment of a neurological or endocrinological disorder. The method comprises exposing a SV2 protein or fragment to the agent and incubating the protein or fragment and agent with (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide. The method further comprises determining if the binding of (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide to the protein is inhibited by the agent, thereby identifying binding partners for the protein.

In another preferred embodiment, the invention includes a method of discovering or modeling an interaction between an SV2 protein, or fragment or derivative thereof, and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site. The method comprises creating a 3-dimensional model of the SV2 protein, or fragments thereof, via either biochemical, biophysical, purely computational techniques, or some combination of these and creating 3-dimensional model of one or a collection of potential ligands that might potentially bind the SV2 protein.

In another preferred embodiment, the invention includes a method of discovering or modeling an interaction between an SV2 protein and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site. The method comprises determining a biochemical, pharmacological, organismal, cellular or molecular effect of a potential CNS active molecule in a genetically wild-type animal or in molecules, cells or tissues derived from such animals

and comparing the measured effect of that compound in an equivalent study in a system with an SV2 protein knocked out or knocked down.

The present invention also provides biotinylated ligands as tools to screen chemical libraries, localize SV2 proteins in tissues, and characterize purified SV2 proteins. SV2 proteins of the present invention includes SV2A, SV2B, and SV2C. Ligands of SV2/LBS, specifically SV2A/LBS, and their derivatives may be biotinylated for screening naturally occurring brain membranes, such as animal, mammalian, or human brain membranes, or for screening cell lines expressing SV2 proteins. The present invention also provides photoactivable biotinylated ligands of SV2/LBS. These screening assays enable the identification of new drugs or compounds that interact with SV2.

Further, the present invention provides a method of purifying a membrane associated protein comprising solubilizing the protein from a tissue to form a solubilized complex and isolating the solubilized complex in a functional form. The solubilized protein or complex may be affinity purified using antibodies that bind to the protein. Examples of membrane associated proteins that may be purified by this method include the family of SV2 proteins such as SV2A, SV2B, and SV2C. The detergents that may be used in the present method includes n-dodecyl-β-maltoside and its analogs or derivatives such as n-octyl, n-nonyl, n-decyl, n-undecyl-β-D-maltoside.

The biotinylated ligands also can be used as tools to assess the conformation state of SV2 proteins after solubilization, immunoaffnity purification, and chromatography.

In one embodiment, the SV2 protein may be a fusion protein comprising at least one SV2 protein or fragment thereof and fusion partner. The fusion partner may be a fusion tag, such as a poly-histidine tag or a glutathione-S-transferase tag. The fusion partner may be attached to the N-terminus or the C-terminus of the SV2 protein.

In another embodiment, the protein, such as the SV2 protein, may lack at least one glycosylation site. In some instances, site-directed mutagenesis may be performed to remove one or more glycosylation site in the SV2 protein.

The SV2 protein or fragment may be purified from natural sources such as mammalian membranes, for example, rat brain membrane. Alternatively, the SV2 protein or fragment is expressed on a transformed host cell. Additionally, the SV2 protein or fragment is immobilized.

In one aspect, the ligand could be directly or indirectly labeled. The label could be a radiolabel, such as ^3H, a fluorescent label, or an enzyme.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the reversible binding of the LEV analog ucb 30889 to LBS in rat brain cortex.

Figure 2 depicts the saturation binding curves of ucb 30889.

Figure 3 shows that specific binding could not be detected in the peripheral tissues.

Figure 4 depicts competition binding curves showing that ucb 30889 binds to LBS with about 10 fold higher affinity than LEV.

Figure 5 depicts pIC_{50} values for ucb 30889 versus levetiracetam.

Figure 6 depicts the concentration dependent inhibition of [^{3}H]ucb 30889 binding by unlabeled levetiracetam in autoradiography of rat brain.

Figure 7 depicts autoradiography of [^{3}H]ucb 30889 binding to coronal sections of rat brain.

Figure 8 depicts the subcellular distribution of [^{3}H]ucb 30889 binding within rat brain.

Figure 9 depicts the subfractionation of the synaptosomal fraction by centrifugation in sucrose gradient.

Figure 10 depicts the photolabelling of the LBS by [^{3}H]ucb 30889 and irreversibility of the complex.

Figure 11 depicts gel electrophoresis of membrane proteins labeled by [^{3}H]ucb 30889.

Figure 12 (A and B) depicts immunostained lysates of the COS-7 cells transfected with SV2A, crude rat brain membranes, and several different PC12 lysates with different levels of LBS.

Figure 13 depicts specific binding of [^{3}H]ucb 30889 to COS-7 transfected with SV2A-12.2, transfected with control β-gal expressing vector, or cells that have not been transfected.

Figure 14 depicts an IC_{50} plot comparing three different ligands binding to SV2A in the presence of 3H-30889.

Figure 15 depicts the structure of (A) levetiracetam and (B) ucb 30889.

Figure 16 (A and B) depicts binding of [^{3}H]ucb 30889 to brain membranes. A. Binding of [^{3}H]ucb 30889 to brain membranes from SV2A, SV2B, and SV2A/SV2B knockout mice. [^{3}H]ucb 30889 alone (□) [^{3}H]ucb 30889 plus 1mM LEV (■). Error bars are the SD of experiments performed with 5 wildtype brains and 4 KO brains. Each experiment was performed in triplicate. B. Western blot of brain membranes from wild type and homozygous knockout mice probed with an anti-SV2 monoclonal antibody (cross-reactive to all isoforms, SV2A, SV2B and SV2C). LANES 1: wt; 2: SV2A ko; 3 SV2B ko; 4: SV2A/B double ko.

Figure 17 (A and B) depicts binding of [^{3}H]ucb 30889 to COS-7 cells expressing hSV2A. A. Binding of [^{3}H]ucb 30889 to hSV2A transiently expressed in COS-7 cells. [^{3}H]ucb 30889 is tested for binding to either untransfected COS-7 cells, or COS-7 cells transiently expressing either □-gal or hSV2A. [^{3}H]ucb 30889 alone (□) [^{3}H]ucb 30889 plus 1mM LEV (■). B. IC_{50} curves of LEV, ucb L060, ucb 30889 against hSV2A transiently expressed in COS-7, in the presence of [^{3}H]ucb 30889. LEV (Δ) ucb 30889 (▪) ucb L060 (•). Error bars are SEM, n=3.

Figure 18 (A and B) depicts binding of [^{3}H]ucb 30889 in the presence of competing drugs. A. Correlation of binding of a series of LEV compounds to mouse brain and to hSV2A, pIC_{50}s measured against [^{3}H]ucb 30889. The pIC_{50} values are the mean of two independent experiments, where each determination lies within 0.2 log units of the mean. B. Correlation of binding of a series of LEV family compounds to hSV2A assayed in transiently transfected COS-7 cells, pIC_{50}s measured against [^{3}H]ucb 30889, and of anti-seizure potencies in the mouse audiogenic model.

Figure 19 depicts the structure of ucb-101282-1. This ligand is a biotinylated derivative of ucb 30889.

Figure 20 depicts that the pKi of ucb-101282-1 is 6.3 (n=2) in rat brain membranes which is equivalent to the affinity reported for LEV.

Figure 21 (A, B, and C) depicts preparation of soluble SV2A and quantitation of by binding assay. A. Detection by western blot using anti-SV2A antibodies of soluble SV2A in the supernatant of solubilized rat brain membranes. B. Analysis of the ability of levetiracetam and ucb 30889 to specifically bind to soluble SV2A. C. Scatchard analysis indicates that the K_D for the binding of [^{3}H] ucb 30889 to SV2A in native rat brain membrane

is 30 nM, while that for the soluble protein is 82 nM.

Figure 22 depicts identification of SV2A partners. Western blot analysis show synaptotagmin associated to soluble SV2A in the immunopurified fractions of the supernatants from solubilized rat brain membranes. The isoform SV2B was not detected.

DETAILED DESCRIPTION

I. Synaptic Vesicle Protein 2 (SV2) Family of Proteins

Any SV2 protein that binds LEV or a derivative or analog thereof may be used in the assays herein described.

As used herein, SV2 proteins include isolated proteins, allelic variants of the proteins, and conservative amino acid substitutions of the proteins. As used herein, the "protein" or "polypeptide" refers, in part, to SV2A, a protein encoded by the nucleic acid sequence of SEQ ID NO: 1 or that has the human amino acid sequence depicted in SEQ ID NO: 2 or fragments thereof; to SV2B, which includes the human protein encoded by the nucleic acid sequence of SEQ ID NO: 3 or the amino acid sequence depicted in SEQ ID NO: 4 or fragments thereof; to SV2C, which includes the human protein encoded by the nucleic acid sequence of SEQ ID NO: 5 or the amino acid sequence depicted in SEQ ID NO: 6 or fragments thereof; and to SVOP, which includes the human protein encoded by the nucleic acid sequence of SEQ ID NO: 7 or the amino acid sequence depicted in SEQ ID NO: 8 or fragments thereof. The terms also refer to naturally occurring allelic variants and proteins that have a slightly different amino acid sequence than that specifically recited above. Allelic variants, though possessing a slightly different amino acid sequence than those recited above, will still have the same or similar biological functions associated with these proteins.

As used herein, the family of SV2 proteins related to the human amino acid sequence of SEQ ID NO: 2, 4, 6 or 8 refers in part, to proteins that have been isolated from organisms in addition to humans. For example, rat homologues of SV2A nucleic acid (SEQ ID NO: 9) and protein (SEQ ID NO: 10), SV2B nucleic acid (SEQ ID NO: 11) and protein (SEQ ID NO: 12), SV2C nucleic acid (SEQ ID NO: 13) and protein (SEQ ID NO: 14) and SVOP nucleic acid (SEQ ID NO: 15) and protein (SEQ ID NO: 16) have been identified and are included herein. The methods used to identify and isolate other members of the family of proteins related to these proteins are described below.

The SV2 proteins used in the present invention are preferably in isolated form in part of a cellular or vesicle membrane fragment, expressed in a transformed host cell, or naturally expressed in a given cell or tissue type. As used herein, a protein is said to be isolated when physical, mechanical or chemical methods are employed to remove the protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated protein.

The SV2 proteins that may be used in the methods of the invention further include insertion, deletion, conservative amino acid substitution or splice variants of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16. As used herein, a "conservative" variant refers to alterations in the amino acid sequence that do not adversely affect the biological functions of the protein. A substitution, insertion or deletion is said to adversely affect the protein when the altered sequence prevents or disrupts a biological function associated with the protein. For example, the overall charge, structure or hydrophobic/hydrophilic properties of the protein can be altered without adversely affecting a biological activity. Accordingly, the amino acid sequence can be altered, for example to render the peptide more hydrophobic or hydrophilic, without adversely affecting the biological activities of the protein. As used herein, a "deletion" is defined as a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent; an "insertion" or "addition" is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring SV2 and a "substitution" results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

SV2 proteins of the present invention further include fusion proteins, wherein a SV2 protein, or fragment thereof, is N- or C- terminally fused to another SV2 protein or fragment thereof, which may be the same as or different from the first SV2 protein or fragment thereof, and/or to a heterologous peptide fusion partner. The heterologous peptide may be a polypeptide sequence useful for the expression, purification, solubility, identification, antigenicity, or extension of the stability of the SV2 protein or fragment thereof. Heterologous fusion partners useful in the present invention include, but are not limited to, glutathione-S-transferase (GST), poly-histidine tags, green fluorescent protein (GFP), albumin, and ovalbumin or fragments thereof.

Ordinarily, the allelic variants, the conservative substitution variants, and the members of the SV2 protein family, will have an amino acid sequence having at least about 35%, 40%, 50%, 60%, 65%, 70% or 75% amino acid sequence identity with the full length sequence set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 more preferably at least about 80%, even more preferably at least about 90%, and most preferably at least about 95%, 97% or 99% sequence identity. Identity or homology with respect to such sequences is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the known peptides, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity (see section B for the relevant parameters). Fusion proteins, or N-terminal, C-terminal or internal extensions, deletions, or insertions into the peptide sequence shall not be construed as affecting homology.

Contemplated variants further include those containing predetermined mutations by, *e.g.*, homologous recombination, site-directed or PCR mutagenesis, and the corresponding proteins of other animal species, including but not limited to rabbit, mouse, rat, porcine, bovine, ovine, equine and non-human primate species, and the alleles or other naturally occurring variants of the family of proteins; and derivatives wherein the protein has been covalently modified by substitution, chemical, enzymatic, or other appropriate means with a moiety other than a naturally occurring amino acid (for example a detectable moiety such as an enzyme or radioisotope).

Fragments of the SV2 proteins may also be used in the methods of the invention. In particular, fragments comprising the LEV binding site may be used. Such fragments may have at least about 6 or 10, 15 or 20, or 25 or 30 amino acid residues, about 35 or 40 amino acid residues, about 45 or 50 amino acid residues, about 55 or 60, about 65 or 70 amino acid residues or at least about 75 or more amino acid residues

The methods of the present invention may also utilize nucleic acid molecules that encode members of the SV2 protein family, including, but not limited to, both the rat and human proteins known as SV2A, SV2B, SV2C and the related synaptic vesicle protein SVOP, such as those consisting of or comprising SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 and the related proteins herein described, preferably in isolated form. Vectors, plasmids and transformed host cells may also be used to produce an SV2 protein. As used herein, "nucleic

acid" is defined as RNA or DNA or related molecules that encodes a protein or peptide as defined above, is complementary to a nucleic acid sequence encoding such peptides, hybridizes to such a nucleic acid and remains stably bound to it under appropriate stringency conditions, or encodes a polypeptide sharing at least about 35%, 40%, 50%, 60%, 65%, 70% or 75% sequence identity, preferably at least about 80%, more preferably at least about 85%, and even more preferably at least about 90%, 95%, 97% or 99% or more identity with the full-length peptide sequence of SEQ ID NO: 2, 4, 6, 8, or 10. The "nucleic acid molecules" useful in the invention further include nucleic acid molecules that share at least about 70% or 75% sequence identity, preferably at least about 80%, more preferably at least about 85%, and even more preferably at least about 90% and most preferably 95%, 97%, 99% or more identity with the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, or 9. Nucleic acids of the present invention also include those which encode fusion proteins comprising a SV2 protein either N- or C- terminally fused to a heterologous protein sequence or to another SV2 protein sequence.

Homology or identity at the nucleotide or amino acid sequence level is determined by **BLAST** (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs **blastp, blastn, blastx, tblastn** and **tblastx** (Altschul, *et al.*, Nucleic Acids Res. 25: 3389-3402 (1997); Karlin *et al.*, Proc. Natl. Acad. Sci. USA 87:2264-2268 (1990)) which are tailored for sequence similarity searching. The approach used by the **BLAST** program is to first consider similar segments, with and without gaps, between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a pre-selected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.*, (Nature Genetics 6, 119-129 (1994)). The search parameters for **histogram, descriptions, alignments, expect** (*i.e.*, the statistical significance threshold for reporting matches against database sequences), **cutoff, matrix** and **filter** (low complexity) are at the default settings. The default scoring matrix used by **blastp, blastx, tblastn,** and **tblastx** is the **BLOSUM62** matrix (Henikoff *et al.*, Proc. Natl. Acad. Sci. USA 89:10915-10919 (1992)), recommended for query sequences over 85 in length (nucleotide bases or amino acids).

For **blastn**, the scoring matrix is set by the ratios of **M** (*i.e.*, the reward score for a pair

of matching residues) to N (*i.e.*, the penalty score for mismatching residues), wherein the default values for M and N are +5 and -4, respectively. Four **blastn** parameters were adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every winkth position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent **Blastp** parameter settings were Q=9; R=2; wink=1; and gapw=32. A **Gap** comparison between sequences, available in the Accelrys' Wisconsin Package version 10.2, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

"Stringent conditions" include those that (1) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C, or (2) employ during hybridization a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C. Another example is hybridization in 50% formamide, 5× SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5× Denhardt's solution, sonicated salmon sperm DNA (50 μg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2× SSC and 0.1% SDS. A skilled artisan can readily determine and vary the stringency conditions appropriately to obtain a clear and detectable hybridization signal. Preferred molecules are those that hybridize under the above conditions to the complement of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15 and which encode a functional protein. Even more preferred hybridizing molecules are those that hybridize under the above conditions to the complement strand of the open reading frame of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15.

As used herein, a nucleic acid molecule is said to be "isolated" when the nucleic acid molecule is substantially separated from contaminant nucleic acid molecules encoding other polypeptides.

A. SV2A and the Levetiracetam Binding Site (LBS)

The invention includes the characterization and use of the LBS located on the SV2A protein.

As described above, "SV2A" includes the human protein as described in SEQ ID NO: 2, the human protein encoded by SEQ ID NO: 1, species homologues of human SV2A, variants of SEQ ID NO: 2 as herein described, and fragments of SV2A comprising the LBS.

II. Levetiracetam and Analogs

The methods of the invention include the use of LEV and LEV analogs or derivatives thereof in assays to identify new pharmacological agents. In a preferred embodiment, the methods of the present invention identify compounds or agents that compete with LEV and LEV analogs or derivatives thereof for binding to the LBS of SV2. As used herein, the terms "compete" and "competitive binding" refer to agents or compounds which occupy the same binding site on the LBS as LEV or analogs or derivatives thereof; displace, or are displaced by, LEV or analogs or derivatives thereof in binding to the LBS; or inhibit, or are inhibited by, LEV or analogs or derivatives thereof in binding to the LBS. In another preferred embodiment, the invention includes the identification of compounds or agents that modulate the activity of SV2A. In another preferred embodiment the methods of the present invention identify compounds or agents which have less, about the same, or greater affinity for the LBS than LEV. In yet another preferred embodiment the methods of the present invention identify compounds or agents which have less, about the same, or greater affinity for the LBS than ucb 30889. In still another preferred embodiment the methods of the present invention identify compounds or agents which in an effective amount modulate the activity of SV2A for a longer period of time than an effective amount of LEV. In even another preferred embodiment the methods of the present invention identify compounds or agents which in an effective amount modulate the activity of SV2A for a shorter period of time than an effective amount of LEV.

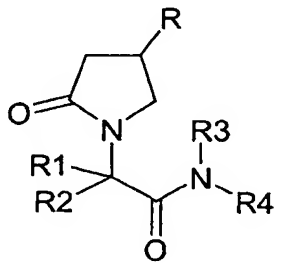
As used herein, "levetiracetam" (Figure 15A; LEV), refers to the International Non-proprietary name of the compound (S)-α-ethyl-2-oxo-1-pyrrolidine acetamide as disclosed in European Patent No. 0 162 036 B1, herein incorporated by reference in its entirety. LEV is a levorotary compound which is a protective agent for the treatment and prevention of hypoxic and ischemic type aggressions of the central nervous system. This compound is also effective in the treatment of epilepsy. Racemic α-ethyl-2-oxo-1-pyrrolidine acetamide and

analogs thereof are known from British Patent No. 1 309 692. US Patent No. 3,459,738 discloses derivatives of 2-oxo-1-pyrrolidine acetamide.

As used herein, the term "LEV analogs or derivatives thereof" includes optionally substituted N-alkylated 2-oxo-pyrrolidine derivatives. Preferably, those compounds are alkyl amides derivatives substituted on the positions 4 and/or 5 of the pyrrolidone ring. Examples of optionally substituted N-alkylated 2-oxo-pyrrolidine derivatives include, but are not limited to, compounds such as those disclosed in international patent application PCT/EP01/01992 such as (2S)-2-[(4S)-4-(2,2-difluorovinyl)-2-oxopyrrolidinyl]butanamide, (2S)-2-[(4R)-2-oxo-4-propylpyrrolidinyl]butanamide, (2S)-2-[(4S)-2-oxo-4-propylpyrrolidinyl]butanamide, and (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide.

As used herein, the term "LEV analogs or derivatives thereof" further include optionally substituted N-alkylated 2-oxo-piperidinyl derivatives. Preferably, those compounds are alkyl amides derivatives substituted on the position 4 and/or 5 and/or 6 of the 2-oxo-piperidinyl ring. Examples of optionally substituted N-alkylated 2-oxo-pyrrolidine derivatives include, but are not limited to, compounds such as those disclosed in international patent application PCT/EP02/05503 such as (2S)-2-[5-(iodomethyl)-2-oxo-1-piperidinyl]butanamide, (2S)-2-[5-(azidomethyl)-2-oxo-1-piperidinyl]butanamide, 2-(2-oxo-5-phenyl-1-piperidinyl]butanamide, (2S)-2-[4-(iodomethyl)-2-oxo-1-piperidinyl]butanamide, and (2S)-2-[4-(2-fluoro-2-methylpropyl)-2-oxo-1-pyrrolidinyl]butanamide.

As used herein, the term "LEV analogs or derivatives thereof" includes any acetam compound of formula I, in racemic or isomeric form, or a pharmaceutically acceptable salts thereof,



(I)

wherein

- R represents hydrogen or hydroxy;
- R^1 and R^2 represent independently hydrogen or an alkyl group of 1-4 carbon atoms; and
- R^3 and R^4 represent independently hydrogen, an alkyl group of 1-4 carbon atoms or $-(CH_2)_n - NR^5R^6$ wherein n is 1, 2 or 3 and R^5 and R^6 represent independently hydrogen or an alkyl group of 1-4 carbon atoms.

An example of such an acetam compound includes, but is not limited to, a compound of formula I wherein R, R^1, R^2, R^3 and R^4 are hydrogen, 2-oxo-pyrrolidineacetamide, known by the generic name piracetam as described in UK Patents Nos. 1,039,113 and 1,309,692.

As used herein, the term "LEV analogs or derivatives thereof" also include optionally substituted N-alkylated 2-oxo-azepanyl derivatives. Preferably, those compounds are alkyl amides derivatives substituted on the positions 4 and/or 5 and/or 6 and/or 7 of the 2-oxo-azepanyl ring. Examples of optionally substituted N-alkylated 2-oxo-azepanyl derivatives include, but are not limited to, compounds such as those disclosed in international patent application PCT/EP02/05503 such as 2-[5-(iodomethyl)-2-oxo-1-azepanyl]butanamide.

In another embodiment the present invention includes compounds or agents which are derivatives or analogs of piracetam which bind to the LBS. Such compounds would also include molecules such as aniracetam and nefiracetam. In a preferred embodiment, the derivatives or analogs of piracetam are those which modulate the activity of SV2A or other SV2 family members.

III. Assay Formats

Assays of the present invention include methods of identifying agents or compounds which are useful for the treatment of neurological disorders, such as seizures, epilepsy, Parkinson's disease, Parkinson's dyskinesias, migraine, Alzheimer's disease, neuropathic pain, essential tremor, cognitive disorders, movement disorders, endocrinopathy and adrenal-medulla-related disease, such as hypoglycemia and circulation shock. Assays of the present invention also include methods of identifying agents or compounds which have cognitive enhancing effects, such as for example might be measured in animal models of cognition. In particular, the assays of the present invention include methods of identifying agents or

compounds that compete with LEV or analogs or derivatives thereof for binding to the LBS of SV2A, displace, or are displaced by, LEV or analogs or derivatives thereof in binding to the LBS; or inhibit, or are inhibited by, LEV or analogs or derivatives thereof in binding to the LBS.

LEV, ucb 30889 (Figure 15B)and other derivatives or analogs of LEV as described above are useful in the methods of the invention as binders in assays to screen for new compounds or agents that bind to the LBS of SV2A. In such assay embodiments, LEV, ucb 30889 and derivatives or analogs can be used without modification or can be modified in a variety of ways; for example, by labeling, such as covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In any of these assays, the materials can be labeled either directly or indirectly. Possibilities for direct labeling include label groups such as: radiolabels including, but not limited to, [^{3}H], [^{14}C], [^{32}P], [^{35}S] or [^{125}I], enzymes such as peroxidase and alkaline phosphatase, and fluorescent labels capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization, including, but not limited to, fluorescein or rhodamine. In addition, FRET techniques could be used to analyze interactions between ligands and the LBS of SV2A. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups or the use of anti-ligand antibodies. The compounds may also include spacers or linkers in cases where the compounds are to be attached to a solid support.

To identify agents or compounds which compete or interact with LEV and ucb 30889 and derivatives for binding to the LBS of SV2A, intact cells, cellular or membrane fragments containing SV2A or the entire SV2A protein or a fragment comprising the LBS of the SV2A protein can be used. The agent or compound may be incubated with the cells, membranes, SV2 protein or fragment prior to, at the same time as, or after incubation with LEV or an analog or derivative thereof. Assays of the present invention can measure any property or function known for SV2 proteins, synaptic vesicles, neural transmission and/or endocrine cell function, as well as presynaptic accumulation of divalent cations, including Ca^{2+}. Examples of properties or functions of an SV2 protein which may be measured as an assay endpoint include, but are not limited to, phosphorylation state, binding of divalent cations, including Ca^{2+}; membrane transport; transport of divalent cations (including Ca^{2+}) into and/or out of

synaptic vesicles; transport of neurotransmitters (including, but not limited to amines, acetylcholine, excitatory neurotransmitters, GABA, serotonin, and glycine) into and/or out of synaptic vesicles; interaction with other proteins (including, but not limited to laminins and synaptotagmin); conformational changes, as measured by sensitivity to proteolysis or other changes in biochemical or biophysical properties; divalent cation channel formation; formation or dissociation of protein complexes; synaptic vesicle function; fusion; exocytosis; and synaptic vesicle recycling.

Assays of the invention may be modified or prepared in any available format, including high-throughput assays that monitor the binding of LEV or the binding of derivatives or analogs thereof to SV2A or to the LBS of the SV2A protein. In many drug screening programs which test libraries of compounds, high throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Such screening assays may use intact cells, cellular or membrane fragments containing SV2A as well as cell-free or membrane-free systems, such as may be derived with purified or semi-purified proteins. The advantage of the assay with membrane fragment containing SV2A or purified SV2A proteins and peptides is that the effects of cellular toxicity and/or bioavailability of the test compound can be generally ignored, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an inhibition of, for instance, binding between two molecules.

In one embodiment of a competitive screening assay, the assay can be formulated to detect the ability of a test agent or compound to inhibit binding of ucb 30889 to SV2A or a fragment of SV2A comprising the LBS or of LEV, or derivatives or analogs thereof, to SV2A or a fragment of SV2A comprising the LBS. In another embodiment of a competitive screening assay, the assay can be formulated to detect the ability of ucb 30889 or of LEV, or derivatives or analogs thereof, to inhibit binding of a test agent or compound to SV2A or a fragment of SV2A comprising the LBS. The inhibition of complex formation may be detected by a variety of techniques. For instance, modulation of the formation of complexes can be quantitated using, for example, detectably labeled ucb 30889, LEV, or derivatives or analogs of LEV. The inhibition of complex formation may be detected by using a detectably labeled version of the agent or compound being assayed for competitive binding to the LBS of SV2A. Alternatively, the binding between the SV2A protein and a ligand may be detected

with no need of a labeled probe. For instance surface plasmon resonance, nuclear magnetic resonance or mass spectrometry are the instruments of choice for such binding assays. Another method is to measure changes in the sensitivity of SV2 proteins to proteases induced by binding of a ligand.

In certain instances, it will be desirable to immobilize one of the LBS (SV2A or a fragment of SV2A comprising the LBS) or the ligand (LEV, ucb 30889 or the test agent or compound) to facilitate separation of complexes from uncomplexed forms, as well as to accommodate automation of the assay. Binding of a ligand to the LBS, for instance binding of a candidate agent or compound to SV2A, in the presence and absence of LEV or ucb 30889, can be accomplished in any vessel suitable for containing the reactants. Examples include microtiter plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion protein can be provided which adds a domain that allows the LBS to be bound to a matrix. For example, glutathione-S-transferase(GST) fusion proteins can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtiter plates, which are then combined with the labeled LEV, ucb 30889, or derivatives or analogs of LEV and the unlabeled test agent or compound; or alternatively, with the unlabeled LEV, ucb 30889, or derivatives or analogs of LEV and the labeled test agent or compound. The mixture is then incubated under conditions conducive to complex formation. Following incubation, the beads are washed to remove any unbound reactants, and the matrix immobilized label determined directly, or in the supernatant after the LBS/ligand complexes are subsequently dissociated. When amenable, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of ligand found in the bead fraction quantitated from the gel using standard electrophoretic techniques.

Other techniques for immobilizing proteins on matrices are also available for use in the subject assay. For instance, the LBS can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with the LBS but which do not interfere with ligand binding can be derivatized to the wells of the plate, and LBS binding trapped in the wells by antibody conjugation. As above, preparations of a ligand and a test compound are

incubated in the protein-presenting wells of the plate, and the amount of protein/ligand complex trapped in the well can be quantitated. Exemplary methods for detecting such complexes, in addition to those described above, include immunodetection of complexes using antibodies reactive with the ligand, or which are reactive with the protein and compete for binding with the ligand.

In another embodiment of the invention, competitive binding assays can be carried out using cellular extracts of cells or tissues that comprise the LBS to identify SV2 binding partners. As used herein, a cellular extract refers to a preparation or fraction that is made from a lysed or disrupted cell. The preferred source of cellular extracts will be cells derived from human central nervous tissue or endocrine tissues. In particular, cellular extracts may be prepared from a particular region, including, but not limited to, the hippocampus, the cerebellum, the cerebrum, the cerebral cortex, the pituitary, the medulla, and the adrenal gland. Further, cellular extracts may be prepared from a particular primary cell isolate of central nervous system origin or the endocrine systems including, but not limited to, neurons, astrocytes, and endocrine cells of the medulla. Alternatively, cellular extracts may be prepared from available cell lines, particularly cell lines of a neurological or endocrine origin. Cell lines contemplated herein include, but are not limited to, rat PC12 pheochromocytoma cells, AtT-20, GH3 and HIT cells.

A variety of methods can be used to obtain an extract of a cell. Cells can be disrupted using either physical or chemical disruption methods. Examples of physical disruption methods include, but are not limited to, sonication and mechanical shearing. Examples of chemical lysis methods include, but are not limited to, detergent lysis and enzyme lysis. A skilled artisan can readily adapt methods for preparing cellular extracts in order to obtain extracts for use in the present methods.

Once an extract of a cell is prepared, the extract is mixed with SV2 protein or fragment and other components of the assay under conditions in which association of the protein with the binding partner can occur, followed by the addition of LEV or an analog or derivative thereof. Alternatively, the LEV or an analog or derivative thereof may be added to the cellular extract before or at even time with the test agent or compound. A variety of conditions can be used, the most preferred being conditions that closely resemble conditions found in the cytoplasm of a human cell. Features such as osmolarity, pH, temperature, and

the concentration of cellular extract used, can be varied to optimize the association of the protein with the binding partner.

After mixing under appropriate conditions, the bound complex is separated from the mixture. A variety of techniques can be utilized to separate the mixture. For example, antibodies specific to SV2A can be used to immunoprecipitate the binding partner complex. Alternatively, standard chemical separation techniques such as chromatography and density/sediment centrifugation can be used.

After removal of non-associated cellular constituents found in the extract, the binding partner can be dissociated from the complex using conventional methods. For example, dissociation can be accomplished by altering the salt concentration or pH of the mixture.

As discussed above, to aid in separating associated binding partner pairs from the mixed extract, the LBS can be immobilized on a solid support. For example, the LBS can be attached to a nitrocellulose matrix or acrylic beads. Attachment of the LBS to a solid support aids in separating peptide/binding partner pairs from other constituents found in the extract. The identified binding partners can be either a single protein or a complex made up of two or more proteins. Alternatively, binding partners may be identified using a Far-Western assay according to the procedures of Takayama *et al.* (Methods Mol. Biol. 69:171-184. (1997)) or Sauder *et al.* (J. Gen.Virol. 77:991-996. (1996)) or identified through the use of epitope tagged proteins or poly-His fusion or GST fusion proteins.

Alternatively, mammalian cell-based protein-protein assays utilizing bioluminescence or fluorescence energy transfer (BRET and FRET, respectively) and the yeast two-hybrid system may be a tool for the identification of protein-protein interactions.

Another approach to identifying pharmacologically active compounds that act via the SV2 proteins is by analyzing the effects of such compounds on wild-type and SV2 knockout cell lines, tissues, and animals. For example, compounds of interest, which might have previously been identified by testing in genomic wild-type animal or tissue models of disease, or by screening against functional cellular assays, can be re-tested in equivalent or informative assays in cells, tissues or animals that have reduced or low levels of functional SV2 proteins, or which lack functional SV2 proteins altogether. Such knockdowns or knockouts might be obtained, for example, by using anti-sense or RNAi techniques, or by working with genomic knockout animals.

In some embodiments, compounds that inhibit N-type calcium channels in neurons of wild-type animals are identified, followed by testing the compounds under the same conditions in neurons that have their SV2 proteins knocked down using RNAi or antisense oligos targeted to the SV2 mRNA sequences, or, alternately, neurons from genomic SV2 knockout animals. The lack of an effect in the SV2 knockout neurons would be evidence that the compounds are having their effect via SV2 proteins.

In another embodiment, compounds with anticonvulsant properties are identified by testing their ability to inhibit epileptiform field potentials recorded in the CA3 area of wild-type rat hippocampal slices bathed in an epileptogenic medium containing increased potassium and lowered calcium. Compounds that exhibit anticonvulsant properties could then be tested in the same assay using SV2 knockout or knockdown hippocampal slices. If a lack of efficacy was observed in the slices without SV2 protein expression, this would strongly support an effect mediated by interactions with SV2 proteins.

In another embodiment, the effect of compounds or agents which bind to the LBS on presynaptic divalent cation storage can be studied in knockout or knockdown mice. In a particular embodiment, wild-type and SV2 knockout or knockdown mice are administered an amount of the compound or agent which binds to the LBS. Animals are sacrificed and brains are immediately removed and flash-frozen. Elemental imaging of thin freeze-dried cryosections is carried out and the elemental composition of the presynaptic nerve terminals is determined by electron probe x-ray microanalysis and elemental imaging of characteristic x-rays. An example of such a method is disclosed by Andrews *et al.* (Proc. Natl. Acad. Sci. USA 84(6):1713-1717 (1987)).

IV. *In Vitro* Characterization of SV2

The invention includes the functional characterization of the SV2 family of proteins. In one embodiment, the invention includes the cloning and expression of both the rat and human forms of the SV2 proteins SV2A, SV2B, SV2C and the related synaptic vesicle protein SVOP. In another embodiment, the invention further includes identification of the domain or domains comprising the LBS. In an additional embodiment, the invention includes discovery of possible multiple functions of the SV2 proteins, and of the effect(s) of levetiracetam and related ligands on these functions.

In an additional embodiment, the invention includes expression of the SV2 protein in a eukaryotic host cell for study of function. The protein might be expressed in it's native form, or as fusions with fluorescent or other peptidic tags, including epitope and affinity tags; mutant forms, or fragments of the protein might be expressed and studied, fusions between the protein and homologous proteins might be expressed and studied. The heterologously expressed SV2 might be studied in-situ using electrophysiology, microscopy, or other techniques; or it might be expressed and purified in functional form from the eukaryotic using electrophysiology or other techniques.

In one particular embodiment, the SV2 protein, native or modified as above, might be expressed in a eukaryotic host and purified. The protein might be purified and incorporated into artificial lipid vesicles, or artificial bilayer membranes for study. Possible transport functions of the SV2 protein might be studied by biochemical means, for instance measuring radioactively labeled substrate transport into or out of the vesicles. Another possible approach is to use electrophysiology to study such transport in purified protein incorporated into synthetic vesicles or artificial lipid membranes.

In another particular embodiment, the invention includes the expression of the SV2 protein in a prokaryotic host, such as *E. coli*, and purification. In another particular embodiment, the invention includes the recombinant expression of the SV2 protein in a eukaryotic host, including yeast (*Saccharomyces cerevisie or Pichia pastoris, for example*), COS-7, HEK293 and PC12a cells, and purification. In accordance with the present invention, polynucleotide sequences that encode SV2 proteins, fragments of the polypeptide, fusion proteins or functional equivalents thereof may be used to generate recombinant DNA molecules that direct the expression of SV2 protein in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be used to clone and express SV2. As will be understood by those of skill in the art, it may be advantageous to produce SV2-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray *et al.* Nuc. Acids Res. 17:477-508. (1989)) can be selected, for example, to increase the rate of SV2 expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

In another embodiment, SV2 proteins of the invention are recombinantly co-expressed in host cells with other proteins SV2 is normally associated with in synaptic vesicles. In a preferred embodiment, SV2 proteins are co-expressed with SNARE complex proteins including vesicle-associated VAMP/synaptobrevin, syntaxin and SNAP-25. In a preferred embodiment, SV2A is recombinantly co-expressed in a host cell with recombinantly expressed synaptotagmin.

In another embodiment, the roles of glycosylation, phosphorylation and other natural or introduced protein modifications in SV2 protein function, stability and interaction are analyzed. Nucleotide sequences encoding SV2 proteins of the present invention can be engineered using methods generally known in the art in order to alter sequences encoding SV2 proteins for a variety of reasons, including but not limited to; alterations which modify the cloning, processing, and/or expression of the gene product; alterations which modify the interaction of SV2 proteins with binding partners; alterations of the solubility and/or membrane insertion of SV2 proteins; and alterations which affect the LBS and its association with ligands. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, site-directed mutagenesis may be used to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to produce splice variants, or to introduce mutations, and so forth.

Upon exocytosis at the synapse, vesicles cluster at the pre-synaptic plasma membrane and fuse in response to increased Ca^{2+} levels. Upon Ca^{2+} accumulation within the synapse, the binding of synaptotagmin to SV2A is inhibited and dimerization of two synaptotagmin Ca^{2+}-binding domains (C2B) is stimulated, which may play a role in organizing the SNARE complex and promoting fusion. At low Ca^{2+}, the fusion of vesicles is inhibited because SV2A is still attached to the synaptotagmin complex. Binding of synaptotagmin to other proteins, including the ATPase VCP, the SNARE protein SNAP-25 and syntaxin, is Ca^{2+}-dependent (Augustine, 2001). In order to shed light on this exocytosis mechanism and define more precisely the role of SV2A in the fusion process, changes in protein levels within these complexes in response to modulation of the LBS are assayed. In particular embodiments, the ability of LBS ligands to modulate the interactions between SV2A and the synaptotagmin-SNARE complex and to assess which stage of the complex assembly and which partners are

modulated by the binding to SV2A of LEV, analogs or derivatives thereof, or compounds or agents which compete with LEV for binding to the LBS. In one such embodiment, protein stoichiometry in the complex after ligand addition is analyzed using antibodies specific for the identified SV2A partners and a combination of immunoprecipitation and recombinant GST-fusion protein affinity chromatography.

In another such embodiment, mass spectrometry and/or surface plasmon resonance are used to detect the effects of LBS ligands on the interactions between SV2A and its partner (*e.g.* synaptotagmin) or short peptides derived from binding domains. In another particular embodiment, biochemical approaches are used to demonstrate if LBS ligands compete with bivalents (such as Ca^{2+}, Pb^{2+}, Zn^{2+}) and inhibit their interactions with SV2A and/or synaptotagmin. In another particular embodiment, the role of the SV2 proteins in synaptic vesicle fusion and recycling is analyzed by the creation of PC12a cell lines, primary neuronal cultures, chromaffin cells and other cell lines or primary isolates expressing fusion constructs between the SV2 proteins and GFP. In one such embodiment, these cell lines are analyzed by fluorescence microscopy tracking of SV2 complexes and synaptic vesicle exocytosis and trafficking, and the effects of treatments with LBS ligands on these events. The cell types described above can also be used to measure vesicle fusion and exocytosis (using encapsulated dye into the vesicles or measuring the release of labeled neurotransmitters) and the ability of LBS ligands to modulate these activities.

In other embodiments of characterizing SV2 proteins and its binding partners, enrichment of the entire multi-protein complex is achieved by affinity-based methods using GST-fusion SV2 or anti-SV2 antibodies. In a particular embodiment, SV2A is overexpressed in PC12 cells with a GST tag and, together with its partners, immunoprecipitated by an antibody against the tag. In a related embodiment, SV2A is immobilized onto agarose beads using a GST or poly-histidine tag. In a preferred embodiment, synaptic vesicle extracts, cell extracts or brain extracts are incubated with the beads, SV2A is cleaved off and eluted proteins are resolved by 1D or 2D gels and analyzed. In a further embodiment, identification of these proteins is used to search databases for novel putative interacting partners. In another embodiment, the yeast two-hybrid (Y2H) system or mammalian cell-based protein-protein assays are used for the identification of protein-protein interactions within living organisms to confirm SV2 binding partners found by the affinity-based methods and to define

the specific protein domain interactions using known cDNAs.

Antibodies specific for SV2 proteins may be produced by inoculation of an appropriate animal with the polypeptide or an antigenic fragment. Antibodies specific for the LBS may be produced by inoculation with full-length SV2 protein or a fragment comprising the LBS. An antibody is specific for the particular SV2 if it is produced against an epitope of the polypeptide and binds to at least part of the natural or recombinant protein. Monoclonal and/or polyclonal antibodies specific for SV2 or for the LBS may be produced by any of a number of methods which are well known in the art for antibody production, such as those taught by Harlow and Lane (Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. (1988)). SV2 peptides for antibody induction do not require biological activity; however peptides must be immunogenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acids, preferably at least 10 amino acids. They should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of SV2 amino acids may be fused with those of another protein such as keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Antibody production includes not only the stimulation of an immune response by injection into animals, but also analogous steps in the production of synthetic antibodies or other specific-binding molecules such as the screening of recombinant immunoglobulin libraries (see *e.g.* Orlandi *et al.* Proc. Nat. Acad. Sci. USA 86:3833-3837. (1989); Huse *et al.* Science 256:1275-1281. (1989)) or the *in vitro* stimulation of lymphocyte populations. Current technology (Winter G. and Milstein C. (1991) Nature 349:293-299) provides for a number of highly specific binding reagents based on the principles of antibody formation. These techniques may be adapted to produce molecules specifically binding SV2 or the LBS.

In a particular embodiment, the present invention includes the human SV2C protein of SEQ ID NO: 6 and the nucleic acid molecule encoding it (SEQ ID NO: 5), as well as allelic variants and functional equivalents thereof. The invention further includes identification of the *in vivo* distribution of the SV2C protein, including, but not limited to, within the central nervous system, peripheral nervous system and endocrine cells and tissues. The invention further includes identification of ligands and/or binding partners of the SV2C

protein. The invention further includes elucidation of the function of the SV2C protein.

V. SV2 Expression in Disease

The invention includes elucidating the expression of SV2 proteins in relation to specific neurological diseases. In a particular embodiment, antibodies specific for SV2A are used to probe brain tissue in a regional specific manner within the brain, spinal cord and neuroendocrine tissues or cells such as chromaffin cells of control animals and animals mimicking epilepsy, epileptogenesis, Parkinson's disease and cognition deficits, other CNS disorders (see above) and endocrinopathy and adrenal medulla-related diseases. In another embodiment, the invention includes the elucidation of the relationship of all SV2 protein isoforms to the pathologies described above, including alterations or switching of isoforms. In a preferred embodiment DNA microarrays are probed for the expression of SV2 protein coding sequences, and changes thereof, in relation to different neurological diseases. An example of using DNA microarrays for determining expression of a particular nucleic acid sequence can be found in U.S. Patent 5,900,882. In another preferred embodiment, changes in regional or global SV2 protein expression in relation to a neurological disorder associated with synaptic vesicle function is validated by quantitative PCR (qPCR).

In another embodiment, knockout mice are analyzed for the presence of the LBS. In a preferred embodiment, purified synaptic vesicles from mice with the lethal SV2A knockout phenotype, SV2B or double KO SV2A/B are purified and analyzed for the presence of the LBS and for substrate and/or ion uptake in comparison with synaptic vesicles from wild-type mice.

In a particular embodiment, comparisons are made of protein expression levels in synaptic vesicles purified from healthy and diseased animals including, but not limited to, pathologies described above, for example, for protein mapping of synaptic vesicles for the detection of disease-related proteins. In a particular embodiment, comparison of 1D and/or 2D gels of synaptic vesicles derived from the healthy and the diseased states are used to identify proteins that are up- or down-regulated in a disease-specific manner. In another embodiment, targets are identified by comparison of the proteome of synaptic vesicles from wild-type with that of SV2 knock-out mice or double-stranded RNA-induced interference (RNAi; Krichevsky *et al.*, Proc. Nat. Acad. Sci. USA 99(18):11926-11929. (2002)) cultured

neurons. In another embodiment, the invention includes performing RNAi or antisense nucleotides in primary neuronal cultures, cultured neurons or PC12 cells to inhibit or eliminate SV2 expression.

VI. Determination of the LBS Location on the SV2 Protein

A number of methods are employed in the determination of the location of the LBS. The LBS may be comprised of a contiguous segment of amino acid residues, or it may be a 3 dimensional structure comprised of amino acid sequences present on one or more extracellular or intracellular loops or domains. In addition, the LBS may be dependent upon the glycosylation of the SV2 protein or may not require glycosylation of the SV2 protein.

In a particular embodiment, radioligands are used to specifically photoaffinity-label the LBS. In a particular embodiment, the site of covalent attachment of the radioligand is determined by purifying and sequencing the proteolytic fragment from photoaffinity labeled synaptic vesicles with SV2A-antibody affinity chromatography or immunoprecipitation and mass spectrometry.

In particular embodiments for the identification of protein domains involved in the interactions between LBS ligands and the SV2A protein fragments of SV2 proteins, or SV2 proteins with amino acid deletions, additions or substitutions are analyzed for effects on binding. In a preferred embodiment, selected residues will be modified by site directed mutagenesis of the cDNA. In another embodiment, domains are exchanged between SV2 isoforms and structural features of isoforms that are important for ligand recognition are identified. In an example of this embodiment, the N-terminal domain of SV2A is replaced with the shorter equivalent region of SV2B to determine the effect on LBS ligand binding. In another example of this embodiment, a series of swaps are made between regions of SV2A and regions of SVOP, to determine the effect on ligand binding. Such swaps might include large regions of each protein, containing for example, multiple transmembrane regions, as well as small regions of the protein, including for example individual transmembrane regions.

In another embodiment, the three-dimensional structure of the SV2 protein (or selected binding domains) is analyzed using NMR spectroscopy or x-ray crystallography or circular dichroism or infrared spectroscopy utilizing pure SV2A with at least binding activity maintained for the revelation of resolution of the topology of LBS sites and design of new

drugs to fit that receptor. If the binding domain upon investigation requires an hydrophobic environment then the protein must be solubilized in a detergent such as dodecylmaltoside or derivatives (see Examples). Purified protein can be crystallized by methods known in the art, for example, by methods disclosed by A. McPherson in "Preparation and Analysis of Protein Crystals" (John Wiley and Sons, New York, (1982)). Alternatively, SV2 proteins of the present invention may also be crystallized by vapor diffusion and vapor diffusion apparati used in the art may be readily employed in the processes of the present invention. Such apparati are disclosed in, for example, U.S. Pat. Nos. 4,886,646; 5,096,676; 5,130,105; 5,221,410 and 5,400,741, the disclosure of which are herein incorporated by reference. X-ray crystallography determination of SV2 protein structure as well as its association with ligands and/or binding partners can be performed using methods and imaging systems as disclosed in U.S. Patent No. 5,978,444, for example.

In some embodiments, SV2 proteins, including isoforms SV2A, SV2B are SV2C, are recombinantly expressed in host cell lines to screen a diverse set of compounds or agents in binding assays for each isoform. Compounds or agents that interact with the SV2A isoform are analyzed for interaction with other SV2 isoforms. In another embodiment, binding experiments are performed to test several reference drugs, AEDs, steroids and to compare the kinetics of binding between native LBS, human and rat recombinant SV2A.

VII. Uses for Agents on the Invention

The invention includes the use of the compounds or agents identified by methods of the invention for the modulation of SV2 protein. Compounds or agents of the invention can be used to modulate synaptic vesicle function; in particular to modulate disorders associated with synaptic vesicle function, or disorders which might be improved by affecting some aspect of synaptic vesicle function, or also to modulate synaptic vesicle function in order to correct disorders of pre-synaptic function, or disorders of neuronal signaling that can be fixed by compensatory changes in synaptic vesicle function. As used herein, a compound or agent is said to modulate synaptic vesicle function if it is capable of up- or down-regulating at least one function of at least one component of a synaptic vesicle, or the pre-synaptic systems which synaptic vesicles interact with.

In a preferred embodiment, the agent or compound is LEV or an analog or derivative

thereof. In another preferred embodiment, the compound or agent binds to the levetiracetam binding site of an SV2 protein. In still another preferred embodiment, the compound or agent competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site. In yet another preferred embodiment, agents of the invention for the treatment of neurological disorders include N-alkylated 2-oxo-pyrrolidine derivatives, N-alkylated 2-oxo-piperidinyl derivatives, and N-alkylated 2-oxo-azepanyl derivatives as described above.

In a preferred embodiment, the compound or agent is an anti-SV2 antibody or fragment thereof, including those that bind to the levetiracetam binding site of SV2 protein and may be a polyclonal antibody or a monoclonal antibody. In related preferred embodiments, the antibody fragment is an Fab fragment, Fab' fragment, $F(ab')_2$ fragment or an scFv fragment, whereas the monoclonal antibody is a chimeric antibody, humanized antibody, or a human antibody.

In a preferred embodiment, the invention includes the modulation at least one function or activity of a SV2 protein in a cell, for example, by exposing the cell to a compound or agent that binds to the levetiracetam binding site of the SV2 protein. In particular embodiments, modulation at least one function or activity of a SV2 protein in a cell includes exposure of the cell to the compound or agent *in vitro*, *in vivo*, *in situ* and *ex vivo*. As used herein, modulation of a function of SV2 includes, but is not limited to modulation of the transport of ions or other natural substrates across the membrane of the synaptic vesicle, modulation of the binding of an SV2 protein to a natural ligand thereof, modulation of the binding of an SV2 protein to a binding partner as described above, and remodulation of synaptic vesicle formation, fusion, regulation or function.

In a preferred embodiment, the modulation of SV2 protein in a cell includes modulating synaptic vesicle function in the cell. As used herein, synaptic vesicle functions which may be modulated by compounds or agents identified by the methods of the invention include, but are not limited to, formation of synaptic vesicles in the presynaptic neuron, fusion of synaptic vesicles with other synaptic vesicles or the synaptic membrane, recycling or turnover of synaptic vesicles, association of synaptic vesicles with the presynaptic grid, and neurotransmitter release, association with proteins from the extracellular matrix (laminin-1, etc.) and post-synaptic densities.

In particular embodiments, exposure of the cell to a compound or agent of the invention which modulates at least one function or activity of a SV2 protein in a cell is carried out under conditions where the concentration of monovalent and/or divalent cations in the environment of the cell is controlled. In preferred embodiments, the divalent cation is at least one of Ca^{2+}, Zn^{2+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Cu^{2+}. In preferred embodiments, the monovalent cation is K^{+}. In a particular embodiment, exposing the cell to a compound or agent which binds to the levetiracetam binding site is carried out under conditions with a low monovalent and/or divalent cation concentration, or less than about 1 μM . In another particular embodiment, exposing the cell to a compound or agent which binds to the levetiracetam binding site is carried out under conditions with a physiological monovalent and/or divalent cation concentration, or between about 1 μM and about 1000 μM. In yet another particular embodiment, exposing the cell to a compound or agent which binds to the levetiracetam binding site is carried out under conditions with a high monovalent and/or divalent cation concentration, or more than at least about 1000 μM.

VIII. Treatment Of Neurological Disorders

Compounds or agents identified by the methods of the invention can be used in an effective amount to treat neurological disorders associated with synaptic vesicle function. In a particular embodiment, treatment with the compound or agent modulates a neurological disorder. In a preferred embodiment, the neurological disorder is a seizure disorder. In another preferred embodiment, the neurological disorder is selected from the group consisting of Parkinson's disease, Parkinson's dyskinesias, migraine, Alzheimer's disease, neuropathic pain, essential tremor, and cognitive disorders. In a highly preferred embodiment, the neurological disorder is epilepsy. In another highly preferred embodiment, treatment with the compound or agent enhances cognitive function.

In a preferred embodiment, the agent or compound is LEV or an analog or derivative thereof. In another preferred embodiment, the compound or agent binds to the levetiracetam binding site of an SV2 protein. In still another preferred embodiment, the compound or agent competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site. In yet another preferred embodiment, agents of the invention for the treatment of neurological disorders include N-alkylated 2-oxo-pyrrolidine derivatives, N-

alkylated-2-oxo-piperidinyl derivatives, and N-alkylated 2-oxo-azepanyl derivatives as described above.

In a preferred embodiment, the compound or agent is an anti-SV2 antibody or fragment thereof, including those that bind to the levetiracetam binding site of SV2 protein and may be a polyclonal antibody or a monoclonal antibody. In related preferred embodiments, the antibody fragment is an Fab fragment, Fab' fragment, $F(ab')_2$ fragment or an scFv fragment, whereas the monoclonal antibody is a chimeric antibody, humanized antibody, or a human antibody.

As used herein, a subject can be any mammal, so long as the mammal is in need of modulation of function or activity of a SV2 protein mediated by a compound or agent identifiable by a method of the invention. The term mammal is defined as an individual belonging to the class Mammalia. The invention is particularly useful in the treatment of human subjects.

As used herein, an "effective amount" is an amount of a substance, compound or agent which is effective to inhibit, reduce, ameliorate, modulate or control at least one symptom or effect of a disease, condition or another administered substance, compound or agent either *in vivo*, *ex vivo*, or *in vitro*. Further as used herein, an "effective amount" is an amount of a substance, compound or agent which is effective to enhance at least one cognitive function *in vivo*.

As used herein, an agent is said to modulate a neurological disorder when the agent reduces the degree or severity of at least one symptom the neurological disorder. For instance, seizures in epilepsy may be prevented; the amplitude, magnitude or severity of seizures may be reduced, or the frequency of the occurrence of seizures may be reduced by the administration of compounds or agents which up- or down-regulate or modulate in some way the expression or at least one activity of a SV2 protein of the invention.

The compounds or agents identified by the methods of the present invention can be provided alone, or in combination with other compounds or agents that modulate a particular pathological process. For example, a compound or agent of the present invention can be administered in combination with other known drugs. As used herein, two agents are said to be administered in combination when the two agents are administered simultaneously or are administered independently in a fashion such that the agents will act at the same time. In a

particular embodiment of the invention, the compounds or agents identified by the methods of the present invention can be provided in combination with compounds or agents that modulate GABAergic pathways in the brain. In another embodiment, the compounds of the invention are administrated together with amantadine for combined treatment of L-DOPA and tardive dyskinesia.

The compounds or agents of the present invention can be administered via parenteral, subcutaneous, intravenous, intramuscular, intraperitoneal, epidural, transdermal, topical, or mucosal routes, or combinations thereof. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. Drug solubility and the site of absorption are factors which should be considered when choosing the route of administration of a therapeutic agent.

The present invention further includes compositions containing one or more compounds or agents which modulate expression or at least one activity of a SV2 protein of the invention. While individual needs vary, determination of optimal ranges of effective amounts of each component is within the skill of the art. Typical dosages comprise about 0.1 to about 100 mg/kg body weight. The preferred dosages comprise about 5 to about 80 mg/kg body weight. More preferred dosages comprise about 10 to about 60 mg/kg body weight. The most preferred dosages comprise about 20 to about 40 mg/kg body weight.

In addition to the pharmacologically active agent, the compositions of the present invention may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically for delivery to the site of action.

The compounds or agents may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension include, for example, sodium carboxymethyl

cellulose, sorbitol, and/or dextran. Liposomes can also be used to encapsulate the agent for delivery into the cell. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

Mucosal routes of administration include, but are not limited to, oral, rectal and nasal administration. Preparations for mucosal administrations are suitable in various formulations. If the compound or agent is water-soluble, then it may be formulated in an appropriate buffer, for example, phosphate buffered saline or other physiologically compatible solutions, preferably sterile. Alternatively, if the resulting complex has poor solubility in aqueous solvents, then it may be formulated with a non-ionic surfactant such as Tween, or polyethylene glycol. Thus, the compounds and their physiologically acceptable solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral, or rectal administration or, in the case of tumors, directly injected into a solid tumor. For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

For oral administration, the pharmaceutical preparation may be in liquid form, for example, solutions, syrups or suspensions, or may be presented as a drug product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-

hydroxybenzoates or sorbic acid). The pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinized maize starch, polyvinyl pyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystallina cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds or agents may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds or agents may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophilic drugs.

In practicing the methods of this invention, the compounds or agents of this invention may be used alone or in combination, or in combination with other therapeutic or diagnostic agents. In certain preferred embodiments, the compounds of this invention may be co-administered along with other compounds or agents typically prescribed for these conditions according to generally accepted medical practice such as anticonvulsives. The compounds of this invention can be utilized *in vivo*, ordinarily in mammals, such as humans, sheep, horses, cattle, pigs, dogs, cats, rats and mice, or *in vitro*.

IX. Gene Therapy

SV2 proteins used in treatment can be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy." In a specific embodiment, nucleic acids comprising sequences encoding SV2 proteins or functional derivatives thereof, are administered to treat, inhibit or prevent a neurological disease or disorder associated with aberrant synaptic function, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded SV2 protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel *et al.*, Clinical Pharmacy 12:488-505. (1993); Wu and Wu, Biotherapy 3:87-95. (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596. (1993); Mulligan, Science 260:926-932. (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217. (1993); TIBTECH11(5):155-215. (1993)). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.*, eds., Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an SV2 protein, said nucleic acid sequences being part of expression vectors that express the SV2 protein or fragments or chimeric proteins thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the SV2 protein coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the SV2 protein coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody nucleic acids (Koller *et al.*, Proc. Natl. Acad. Sci. USA 86:8932-8935. (1989); Zijlstra *et al.*, Nature 342:435-438. (1989)).

Delivery of the nucleic acids into a patient may be either direct, in which case the

patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

In a specific embodiment, gene therapy vectors can be delivered *in vivo* to a subject by, for example, intravenous injection, local administration (see U.S. Pat. No. 5,328,470) or by stereotactic injection (see *e.g.*, Chen *et al.* Proc. Natl. Acad. Sci. USA 91:3054-3057. (1994)). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, *e.g.*, retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

Direct administration of the nucleic acid sequences for expression of encoded SV2 protein *in vivo* can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering them so that they become intracellular, *e.g.*, by infection using defective or attenuated retroviral vectors or other viral vectors (see U.S. Pat. No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu *et al.*, J. Biol. Chem. 262:4429-4432. (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, PCT Publications WO 92/06180 dated Apr. 16,1992 (Wu *et al.*); WO 92/22635 dated Dec. 23, 1992 (Wilson *et al.*); WO 92/20316 dated Nov. 26, 1992 (Findeis *et al.*); WO 93/14188 dated Jul. 22, 1993 (Clarke *et al.*); and WO 93/20221 dated Oct. 14, 1993 (Young)). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression,

by homologous recombination (Koller *et al.*, Proc. Natl. Acad. Sci. USA 86:8932-8935. (1989); Zijlstra *et al.*, Nature 342:435-438. (1989)).

In a specific embodiment, viral vectors that contain nucleic acid sequences encoding an SV2 protein of the invention are used. For example, a retroviral vector can be used (see Miller *et al.*, Meth. Enzymol. 217:581-599. (1993)). These retroviral vectors have been to delete retroviral sequences that are not necessary for packaging of the viral genome and integration into host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen *et al.* (Biotherapy 6:291-302. (1994)), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes *et al.*, J. Clin. Invest. 93:644-651. (1994); Kiem *et al.*, Blood 83:1467-1473. (1994); Salmons *et al.*, Human Gene Therapy 4:129-141. (1993); and Grossman *et al.*, Curr. Opin. in Genetics and Devel. 3:110-114. (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. In a preferred embodiment, adenovirus vectors are used. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky *et al.*, Current Opinion in Genetics and Development 3:499-503. (1993) present a review of adenovirus-based gene therapy. Bout *et al.*, Human Gene Therapy 5:3-10. (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld *et al.*, Science 252:431-434. (1991); Rosenfeld *et al.*, Cell 68:143-155. (1992); Mastrangeli *et al.*, J. Clin. Invest. 91:225-234. (1993); PCT Publication WO 94/12649; and Wang, *et al.*, Gene Therapy 2:775-783. (1995).

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh *et al.*, Proc. Soc. Exp. Biol. Med. 204:289-300. (1993); U.S. Pat. No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a

patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including, but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, Meth. Enzymol. 217:599-618. (1993); Cohen *et al.*, Meth. Enzymol. 217:618-644. (1993); Cline, Pharmac. Ther. 29:69-92. (1985)) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (*e.g.*, hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, endocrine cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, *e.g.*, as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, *etc.* In a preferred embodiment, the cells used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see *e.g.* PCT Publication WO

94/08598, dated Apr. 28, 1994; Stemple and Anderson, Cell 71:973-985. (1992); Rheinwald, Meth. Cell Biol. 21A:229. (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771. (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

X. Uses for Biotinylated Ligands

The present invention provides nonradioactive labelled SV2A/LBS ligands containing a biotin tag. Such biotinylated ligands are useful in screening assays with no radioactive waste and higher throughput.

As an example, biotinylated derivatives of SV2A/LBS ligands can be used in screening assays (*e.g.* binding) with native brain membranes or SV2 expressed in cell lines for the discovery of more potent structures. The amount of biotin tag bound to SV2A can be quantified using streptavidin-fluorescein or avidin derivatives.

Biotinylated ligands are also useful for assessing the conformational state of SV2 after solubilization, immunoaffinity purification, and chromatography.

Moreover, the present invention provides photoactivable versions of the ligands for labeling and detection in biological samples. The photoactivable biotinylated ligands may also be used to localize and purify SV2 from tissues, isolated cells, subcellular fractions and membranes. The photoactivable biotinylated ligands could also be used for SV2 cross-linking and identification of binding domains of LBS ligands.

XII. Solubilizing SV2 and Affinity Purification

The present invention provides a method for solubilizing SV2/LBS proteins comprising treating membranes with a detergent. The membrane proteins solubilized by the present method remain active as evaluated by binding assays and protein-protein interaction studies.

Briefly, the method comprised incubating membranes, as an example rat brain membranes, in solubilization buffer containing the detergent n-dodecyl-β-D-maltoside for

about two hours at about 4°C. The incubated solution was subsequently centrifuged to collect the soluble SV2 protein, specifically the SV2A protein, from the supernatant. Presence of the soluble SV2A protein in the supernatant was confirmed by western blot analysis using anti-SV2A antibodies. The binding activity of the soluble SV2A protein in the supernatant was determined through binding experiments with ligands known to bind SV2A, such as levetiracetam and ucb 30889.

Other detergents such as the analogs of n-dodecyl-β-D-maltoside, for example, n-octyl, n-nonyl, n-decyl, n-undecyl-β-D-maltoside could also be used. In fact, preliminary data confirmed that the soluble protein obtained from solubilizing membranes with these detergents retains its binding activity.

The present invention also provides a method of affinity purification of the soluble SV2 protein and identification of putative SV2A partners. Briefly, affinity purification comprised incubating the supernatants from the solubilized membranes with anti-SV2A antibodies overnight at about 4°C. The mixture was then incubated by rotation with protein A-Sepharose beads in buffer for about an hour at about 4°C. The resin was washed several times with an appropriate buffer and the fractions containing the immunopurified SV2Aprotein were collected.

To detect the presence of binding partners of SV2A after affinity purification, a western blot analysis of the immunopurified fractions was performed to detect the presence of synaptotagmin (Figure 22)

The present invention provides a method to purify a membrane-associated protein comprising solubilizing a membrane sample containing the protein with a detergent to form a solubilized complex and isolating the solubilized complex in a functional form. The detergent could be n-dodecyl-β-D-maltoside or derivatives thereof. The protein can then be isolated using an immunoaffinity technique.

The protein purified by the present method can be used to perform structural studies on the protein such as NMR, X-ray crystallography, Infrared spectroscopy, Circular dichroism and other methods well known in the art. The present invention also provides a method of performing SV2 protein interaction studies and for detecting peptides, molecules, and compounds that inhibit or promote the interactions between SV2 and a putative partner. The present invention can be used to identify SV2 binding partners.

The present invention could be used to solubilize SV2A, SV2B and SV2C membrane associated proteins and to affinity purify them for structural studies and for identifying binding partners.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1. Development of a Levetiracetam Analog for Binding Studies

LEV has been shown to bind to a specific binding site located preferentially in the brain (levetiracetam binding site or LBS : Noyer *et al.*, Euro. J. Pharmacol. 286:137-146. (1995); Gillard *et al.* 2003)). However, [^{3}H]LEV displayed only micromolar affinity for this site, making it unsuitable for in depth characterization. This example describes the binding properties of [^{3}H]ucb 30889, (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide, an analogue of levetiracetam. Binding experiments were conducted on crude rat brain membranes at 4°C as described in Noyer *et al.* (Euro. J. Pharmacol. 286:137-146 (1995)). Incubation time for equilibrium studies was 120 min. For kinetic and competition studies, [^{3}H]ucb 30889 (30 Ci / mmol) was used at a concentration of 1.3 nM in 0.5 ml of a Tris-HCl (pH 7.4) buffer containing 2 mM Mg^{2+}. Localization of the LBS in brain substructures was assessed by autoradiography on 25 μm thick slices incubated under similar conditions. Slides were then washed twice for 10 min at 4°C in 50 mM Tris-HCl (pH 7.4) containing 0.5% BSA, dried and exposed for 3 weeks to [^{3}H]Hyperfilm at –20°C. Non-specific binding (NSB) was determined by the inclusion of 1 mM LEV during the incubation period.

Figure 1 shows that [^{3}H]ucb 30889 binds reversibly to LBS in rat brain cortex. Binding kinetics were biphasic: half-times for association and dissociation were respectively, 3 ± 2 min and 4 ± 1 min for the fast component (25 to 50 % of the sites), and 47 ± 13 min and 61 ± 15 min for the slow component. At 25°C, kinetics increased dramatically and only one component remained.

Figure 2 shows that the saturation binding curves of [^{3}H]ucb 30889 were compatible with the labeling of a homogeneous population of binding sites. K_D and B_{max} were respectively 42 ± 10 nM and 5054 ± 704 fmol / mg protein. The B_{max} being similar to the value estimated using [^{3}H]levetiracetam as radioligand in similar membrane preparations (4718 ± 413 fmol / mg protein).

Specific binding could not be detected in the peripheral tissues examined (Figure 3). The limit of detection under the experimental conditions (150 µg of protein / assay and 1.3 nM of radioligand) was a B_{max} of 200 fmol / mg protein. This suggests that there are at least 25 times more binding sites in the cerebral cortex compared to the periphery.

Competition binding curves showed that ucb 30889 binds to LBS with about 10 fold higher affinity than LEV (Figure 4). The pKi of ucb 30889 (7.1 ± 0.2) agrees well with the K_D of [^{3}H]ucb 30889 as determined by the saturation binding curve (Figure 2). pIC_{50} values for a variety of levetiracetam analogues and other compounds known to interact with the LBS, such as pentylenetetrazol or bemegride (Noyer *et al.*, 1995), were identical whether obtained with [^{3}H]ucb 30889 or [^{3}H]levetiracetam (Figure 5).

Rat brain sections incubated with [^{3}H]ucb 30889 (Figure 6) show that LBS labeled by [^{3}H]ucb 30889 are diffusely localized throughout the brain and that this binding can be inhibited by levetiracetam at concentrations equivalent to those observed in *in vitro* binding (Figure 4).

This example demonstrates through competition binding studies and tissue distribution that ucb 30889 and LEV are both labeling the same sites, namely the LBS which is localized throughout the central nervous system. Compared to LEV, ucb 30889 binds to the LBS with 10 fold higher affinity and with a very low non specific binding. These criteria along with suitable binding kinetics at 4°C made it possible to use this radioligand to perform autoradiography binding studies on brain slices (Figure 6) and to show the anatomical distribution of LBS in rat brain.

Example 2. Cellular and Subcellular Distribution of the LBS

To identify and characterize the LBS *in situ*, [^{3}H]ucb 30889 was used to map the LBS within the brain and to study both its cellular and subcellular distribution. For rat brain autoradiography, 25 µm slices were incubated with 1.3 nM [^{3}H]ucb 30889 for 120 min at 4

°C in 50 mM Tris-HCl buffer (pH 7.4). Binding assays with rat brain membranes and various neuronal cell lines were performed under similar conditions. Non-specific binding was determined by the inclusion of 1 mM levetiracetam in the assay. For photolabeling, membranes were incubated with 40 nM [^{3}H]ucb 30889 for 120 min at 4°C in the same buffer, followed by irradiation with UV-light for 30 min (Fuks *et al.*, Eur. J. Pharmacol. 478:11-19 (2003)).

For rat brain autoradiography, 25 μm slices were incubated with 1.3 nM [^{3}H]ucb 30889 for 120 min at 4 °C in 50 mM Tris-HCl buffer (pH 7.4). Figure 7 shows that ucb 30889 binding sites are heterogeneously distributed in the rat brain. While there is no apparent binding in the white matter there is a high level of binding in the dentate gyrus, the superior colliculus, several thalamic nuclei and in the molecular layer of the cerebellum. Binding is less pronounced in the cerebral cortex, the hypothalamus and the striatum. Abbreviations: cc, corpus callosum; Aca, anteria commissure; ic, internal capsule; Mtg, mamillotegmental tractus; Mt, mammillothalamic tractus; ML, molecular layer; Hi, hippocampus; DG, dentate gyrus; sc, superior colliculus; CG, central grey; Pu, caudate putamen; Pv, paraventricular nucleus; MG, geniculate nuclei; Po hy, posterior hippothalamic areas; Hb, habenula; Pi, piriform cortex.

[^{3}H]ucb 30889 binding in cerebellar granule neurons and PC12 cells showed high levels of specific binding (Table 1). The Kd being similar to the value measured in rat cerebral cortex (42 nM; see Example 1). The same specific binding site could not be detected in primary astrocytes and in a range of CNS-related cell lines and non neuronal cell lines. Abbreviation: nd, not detected.

TABLE 1

Density and affinity of [^{3}H]ucb 30889 binding in various cell types

Cell type	B_{max}	Kd
Rat cerebellar granule neurons	0.7 pmol/mg protein	59 nM
Mouse cortical neurons	1.4 pmol/mg protein	34 nM
Mouse cortical astrocytes nd	nd	
PC12	1.4 pmol/mg	40nM
SK-N-SH	nd	nd
NG108-15	nd	nd
N1E-115	nd	nd
HCN-1a	nd	nd
CHO-K1	nd	nd
COS-7	nd	nd

Rat brain membranes were separated by differential centrifugation (Figure 8). Binding to LBS (8A), muscarinic (8B), NMDA (8C) and peripheral benzodiazepine (8D) receptors was determined using [^{3}H]ucb 30889, [^{3}H]NMS, [^{3}H]MK801 or [^{3}H]PK11195, respectively. This study shows that the levetiracetam binding site is present in crude synaptosomes (P2), microsomal membranes (P3) and is enriched in synaptic vesicles (LP2). In contrast, the other studied receptors are not more abundant in LP2 compared to P2 or P3. P1 is a low speed pellet containing nuclei and large debris.

A fractionation onto a sucrose gradient was used to isolate the subcellular compartments from crude synaptosomes. The LBS was found in purified synaptic membranes but was not present in the 1.2 M sucrose pellet containing the purified mitochondrial fraction (Figure 9). As a control for the purity of the subcellular fractions, the distribution of the muscarinic and the peripheral benzodiazepine receptors was also analyzed. Data are expressed as percentage of the total specific binding.

Crude synaptosomes (P2 fraction) were preincubated with 40 nM [^{3}H]ucb 30889, then

irradiated with UV light and washed. At 0 min 1 mM levetiracetam was added and aliquots were counted at the indicated times (Figure 10A). Nonspecific binding (open symbol) was determined using 1 mM levetiracetam. Figure 10B shows the same experiment, but performed in the absence of UV light irradiation. These results indicate that during UV irradiation the radioligand inserts covalently in the binding domain of the LBS.

Photoaffinity labeling was performed in the absence or in the presence of 1 mM levetiracetam. The proteins were resolved by SDS-PAGE using an acrylamide concentration of 7.5 % (w/w) and the radioactivity was assessed in each slice of the gel. The major site of incorporation occurs at a molecular weight of 93,000 (Figure 11) (Fuks *et al.*, 2003)

In this example it is shown that the [^{3}H]ucb 30889 binding site in rat brain has a unique profile of distribution and does not appear to correlate with any specific neurotransmitter system that is typically associated with epilepsy. This novel binding site is restricted to neuronal cell types and several brain areas. This novel radioligand can be used as a photoaffinity label and binds covalently to a membrane protein of high molecular weight which is mainly located in synaptic vesicles.

Example 3. The LBS is on SV2A

In this example, the biochemical characterization of LBS in rat brain led to studies to identify potential candidate LBS proteins for cloning and binding characterization. Based on the integral membrane nature of the protein, brain specific expression, apparent size, and synaptic vesicle localization, the SV2 protein family was analyzed as a candidate for localization of the LBS. Accordingly, SV2 proteins were cloned and assayed for binding of LBS ligands.

Materials: Levetiracetam and derivatives were synthesized at UCB Pharma (Braine-l'Alleud, Belgium). [^{3}H]ucb 30889, (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide (32 Ci/mmol), was custom labelled by Amersham Biosciences (Roosendaal, The Netherlands). The monoclonal antibody against SV2 proteins developed by Buckley and Kelly (Buckley et al., J. Cell. Biol., 100, 1284-94 (1985)) was obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biological Sciences, Iowa City, IA

52242. This antibody is cross-reactive against all three SV2 isoforms, SV2A, SV2B, and SV2C.

Wild-type and Knockout Mouse Binding Experiments

SV2A knockout mice have been previously reported (Crowder *et al.* Proc. Natl. Acad. Sci. U.S.A. 96, 15268-73 (1999)). The generation of SV2B knockouts will be reported elsewhere. SV2B knockouts were bred with animals heterozygous for the SV2A gene disruption to produce SV2A+/-SV2B-/- breeders which were used to generate SV2A/B knockouts. Wild type C57-Bl6 and SV2 KO mouse brain membranes were prepared for binding assays and the binding reaction was performed as described previously with slight modifications (Gillard *et al.*, Eur. J. Pharmacol. In Press (2003)). Frozen whole brains were homogenized (10 % w/v) in 20 mM Tris-HCl buffer (pH 7.4) containing 250 mM of sucrose (buffer A). The homogenates were spun at 30,000 x g at 4°C for 15 min and the pellets resuspended in the same buffer. After incubation at 37°C for 15 min, the membranes were washed 2 times using the same centrifugation protocol. The final pellets were resuspended in buffer A and stored in liquid nitrogen. Thawed brain membrane proteins (0.1 mg/assay) were incubated 120 min at 4°C in 0.5 ml of a 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM $MgCl_2$, and [^{3}H]ucb 30889 (1.8 nM). At the end of the incubation period, the membrane-bound radioligand was recovered by rapid filtration through GF/C glass fiber filters pre-soaked in 0.1% polyethyleneimine. The membranes were washed with 8 ml of ice-cold Tris buffer (pH 7.4). The total filtration procedure did not exceed 10 s per sample. The filters were dried and the radioactivity determined by liquid scintillation. pIC_{50}s determination was performed by computerized non-linear curve fitting methods (Graphpad Prism® software, San Diego, CA).

For Western blot experiments, aliquots of brain homogenates from the wildtype and knockout animals were extracted at room temperature with SDS-PAGE sample buffer containing BME. Equivalent amounts of each sample (approx. 10μg total protein) were loaded on a 4-12% Tris-Glycine NOVEX gradient gel (Invitrogen Life Sciences) and separated. After transfer to a nitrocellulose membrane and blocking, the blot was probed with a monoclonal cross-reactive to all SV2 proteins (Buckley *et al.*, J. Cell. Biol., 100, 1284-94 (1985)), and an HRP-anti-mouse secondary antibody was used to label the primary. The

blot was developed with luminescent horseradish peroxidase reagents and photographed.

Binding Experiments with [^{3}H]ucb 30889 against heterologously expressed hSV2A

For binding experiments on confluent, transfected cells (Figure 17), cells in 24 well plates were slowly cooled to 4°C and rinsed once with cold phosphate-buffered saline (PBS). PBS was aspirated and binding reagents were added in PBS. In binding experiments, [^{3}H]ucb 30889 was added to all wells at 1.8 nM, in the presence or absence of differing amounts of unlabelled inhibitors. The cells were incubated at 4°C for 2h and the assay was terminated by rinsing the cells 3x rapidly with ice-cold PBS. After a final aspiration, 200µl of 0.1N NaOH was added to lyse the cells, and the samples were counted in scintilation fluid on a beta counter.

For binding experiments on previously frozen transfected COS-7 cells (Figure 18), 2 to $3x10^4$ cells were incubated 120 min at 4°C in 0.2 ml of a RPMI-HEPES 25 mM solution containing [^{3}H]ucb 30889 (1.8 nM) and increasing concentrations of unlabelled competing drugs. The termination of the binding reaction by filtration and radioactivity counting was performed as described above.

Audiogenic Seizure Mouse Model

Anti-seizure activity of LEV and analogues were assessed in sound-susceptible mice by exposing the mice to acoustic stimuli of 90-db, 10 to 20-kHz for 30sec, 60 min following intraperitoneal pretreatment. The reported ED_{50} values were obtained from testing of 4 to 8 groups (n=10) administered different doses and reflect the potency of the compounds for inhibiting clonic convulsions.

Human SV2A was cloned from a human fetal brain cDNA library as a 3609 bp PCR product comprising the coding region and significant flanking regions from the transcript. Using a vector containing the SV2A coding region plus significant flanking DNA as a source, the coding region was PCR amplified without the flanking regions. This product was cloned into a GATEWAY (Invitrogen) donor vector for ease of subcloning. Only the use of a cloning vector with strong transcription stop sites directly upstream of the cloning site resulted in successful cloning of coding-region only SV2A cDNA. This suggests that this product may be toxic to *E coli,* even in small amounts. Sequencing of the final pDONR GATEWAY SV2A clone showed that it had 2 mutations: one silent, and one a Leu-to-Pro

mutation. The non-silent mutation was corrected and sequencing confirmed that the correct, full length human SV2A coding sequence was cloned.

The human SV2A coding region was transferred from the pDONR GATEWAY cloning vector to a pDEST 12.2 Gateway expression vector. This vector has a CMV promoter driving the introduced gene, and an SV40 ori, which allows very high levels of replication in the COS-7 cell line, which contains the large T antigen. In addition, the human SV2A coding region was transferred into a pDEST 40 Gateway expression vector. This vector is very similar to the 12.2 vector above, with a CMV promoter driving expression of hSV2A, and an SV40 ori, and a Neomycin resistance gene.

Initial tests of SV2A expression using the pDEST 12.2 vector was performed in the COS-7 cell line, which had previously been demonstrated successful expression of SV2 proteins. The COS-7 cell line was tested for ^{3}H-30889 binding, with no binding above background observed, and thus no significant, measurable presence of the Levetiracetam binding site (LBS). In addition, a PC12 cell line subclone, PC12a, which is low in LBS, was used to establish a pool of PC12 cells expressing hSV2A under stable antibiotic selection.

Lipofectamine 2000 (Invitrogen) transfection reagent was used to transfect DNA into 90% confluent COS-7 cells. Also, the same reagent was used to transfect the hSV2A containing vector into the PC12a cell line, and selecting for antibiotic resistance. Anti-SV2A polyclonal antibody (CalBiochem) was used to test for expression in either transfected COS-7 cells, or transfected PC12a cells, of the SV2A product. Lysates of the COS-7 cells were collected at 18 hrs after transfection on an SDS-PAGE gel, transferred to a membrane, and probed with a polyclonal antibody against SV2A, in comparison to crude rat brain membranes (Figure 12A). Also shown are lysates from a non-transfected COS-7 cells, non-transfected PC12a cells (low in LBS), PC12bs cells (high in LBS), or PC12a cells transfected with hSV2A (Figure 12B). No labeling of protein bands is observed in the untransfected COS-7 control, while the transfected COS-7 cells show multiple bands, with most density in the range of 80-120 kD, perhaps due to multiple glycosylation states of the expressed protein. In addition, SV2A immunoreactivity is present in the PC12bs and PC12a/hSV2A samples, but largely absent in the low LBS PC12a cells (Figure 12B).

In a binding experiment, specific binding was measured of [^{3}H]ucb 30889 to COS-7 cells that have either been transfected with SV2A-12.2, or as controls, a β-gal expressing

vector, or cells that have not been transfected (Figure 13). Triplicate wells of a 24-well plate were incubated with either 1 nM [^{3}H]ucb 30889 (labeled "Hot"), or [^{3}H]ucb 30889 plus an excess of cold Levetiracetam (50 μM) (labeled "Hot + Cold"). The cells were incubated at 4 °C for 2 hours, and then washed rapidly with ice-cold PBS. The cells were lysed on the plate, transferred to scintillation vials with scintillation fluid and counted for ^{3}H decay emission. These results show that COS-7 cells transfected with SV2A have acquired the capability to specifically bind [^{3}H]ucb 30889. In identical intact cell binding experiments using PC12bs cells, known to express the LBS, a 1.5 to 2-fold difference in CPM between the 'hot' and 'hot+cold' samples is typically seen, as compared to the 5-fold difference seen here.

Further studies characterized the binding of [^{3}H]ucb 30889 to SV2A expressed in COS-7 cells in more detail. COS-7 cells were transfected in a 24-well plate and assayed for binding as above. A series of concentrations of either Levetiracetam or cold 30889 were added in order to generate IC_{50}s for these compounds against SV2A expressed in COS-7 cells (Figure 14). These results indicate that SV2A is functionally equivalent with the binding site for Levetiracetam that has been observed in rat brain and PC12 subclones. The correlation between LBS binding affinity and the anti-seizure properties of Levetiracetam and it analogues, taken together with the preceding observation, provide support that the synaptic vesicle protein SV2A is not only the native binding site for the anti-epileptic compound Levetiracetam, but suggests a link between the function and modulation of the synaptic vesicles by Levetiracetam and it's anticonvulsant properties.

In a separate experiment, heterologous expression experiments were performed to confirm that SV2A alone is solely responsible for the brain binding of LEV. Human SV2A was transiently expressed in the COS-7 cell line, as verified by Western analysis (data not shown), and observed binding to [^{3}H]ucb 30889 that is displaced by excess LEV (Figure 17A). No binding under identical conditions to either untransfected COS-7 cells, or COS-7 cells transfected with a vector encoding β-galactosidase. In experiments testing the ability of unlabeled compounds to displace [^{3}H]ucb 30889 from hSV2A expressed in COS-7 cells, the affinities of ucb 30889, LEV, and LEV's enantiomer, ucb L060, show the same rank order, and similar values (Figure 17B), to those previously reported in studies with rat brain ((Noyer *et al.*, Eur. J. Pharmacol. 286, 137-146 (1995); Gillard et al., 2003). Critically, ucb L060 binds with significantly less affinity to hSV2A than does LEV, which is a key characteristic

of the binding site in brain (Noyer *et al.*, Eur. J. Pharmacol. 286, 137-146 (1995); Gillard et al., 2003In addition, the binding of [^{3}H]ucb 30889 against both hSV2B and hSV2C expressed in the transient COS-7 system were tested. The results indicate no binding above background (data not shown), consistent with the results from knockout mouse binding studies.

Testing the binding of LEV and several analogs to hSV2A expressed in COS-7, revealed that pIC_{50}s are highly correlated (r^2=0.98) with the values obtained in mouse brain (Figure 18A) and rat brain extracts (data not shown). There was also a clear correlation between the affinities of these compounds for hSV2A in COS-7 and the potency of their antiseizure protection in the mouse audiogenic model of epilepsy (r^2 =0.84) (Figure 18B). This data is consistent with a previous report of a correlation between binding of LEV analogs in rat brain and potency in the same model (Noyer *et al.*, Eur. J. Pharmacol. 286, 137-146 (1995)). The binding of other AEDs, including valproate, carbamazepine, phenytoin, ethosuximide, felbamate, gabapentin, tiagabine, vigabatrin and zonisamide was also investigated. None of the AEDs at concentrations up to 100 μM, competed with [^{3}H]ucb 30889 for binding to SV2A (data not shown). This confirms previous binding studies of AEDs against the LEV binding site in rat brain (Noyer *et al.*, Eur. J. Pharmacol. 286, 137-146 (1995); Gillard *et al.*, Eur. J. Pharmacol. 2003)).

Example 4. Assays for Compounds Which Modulate Neurological Disorders, Endocrinopathy and Hormonal Diseases

In order to identify compounds or agents which modulate neurological disorders associated with synaptic function and endocrinological disorders, studies were undertaken to identify additional compounds which compete with LEV and ucb 30889 for binding to the LBS of a SV2 protein.

SV2A transfected COS-7 cells as disclosed in Example 3 are exposed to a potential binding partner or agent. Control cells are exposed to vehicle only, or are exposed to unlabeled ucb 30889 or LEV. Following this exposure, the cells are then incubated with [^{3}H]ucb 30889, as in Example 3, cells are incubated at 4 °C for 2 hours, and then washed rapidly with ice-cold PBS. The cells are lysed, transferred to scintillation vials with scintillation fluid and counted for ^{3}H decay emission.

Compounds which are found to compete with ucb 30889 for binding to the LBS are

subject to further analysis for the ability to modulate seizures in audiogenic-susceptible mice. Audiogenic-susceptible mice are administered an amount of the compound which is comparable to an effective amount of LEV. As a control, identical audiogenic-susceptible mice are administered an effective amount of LEV, or a compound which does not modulate seizures, such as piracetam.

Example 5. Biotinylated Ligands as Tools to Screen Chemical Libraries and Characterize the SV2 Proteins

The present invention dicloses a method of using novel biotinylated ligands as tools to screen chemical libraries and characterize SV2 proteins. The present invention provides nonradioactive-labelled SV2A/LBS ligands containing a biotin tag for screening purposes with no radioactive waste and higher throughput. The present invention also provides a photoactivable version for labelling and SV2A/LBS detection in biological samples.

In this example, the binding of ucb-101282-1 to SV2A/LBS was characterized in rat brain membranes. This molecule is a biotinylated derivative of ucb 30889 (Figure 19). This ligand had a pKi of 6.3 (n=2) in rat brain membranes which was equivalent to the affinity reported for L059 (Figure 20). This ligand was also designed to cross-link the biotin tag to the LBS/SV2A with an azidophenyl motif capable of forming a covalent complex with the protein upon UV light irradiation.

Example 6. Method for Solubilizing SV2A and Affinity Purification

The present invention discloses a method of solubilizing SV2A and affinity purification. The method comprises solubilizing SV2A/LBS proteins which includes treating the membrane with a detergent. The method maintains the activity of the membrane proteins after solubilization as evaluated in binding assays and protein-protein interaction studies.

Preparation of Soluble SV2A and Quantitation by Binding Assay

The rat brain membranes were diluted in a solubilization buffer (Tris-HCl 20 mM pH 7.4, 0.25 M sucrose, protease inhibitors Complete Roche) containing n-dodecyl-β-D-maltoside 15 mM and incubated for two hours at 4°C. Subsequently, the solution was centrifuged at 4°C for one hour at 100,000 g. The soluble SV2A was found in the

supernatant as detected by western blot using anti-SV2A antibodies (Figure 21A). The supernatants were incubated with [^{3}H]ucb 30889 as described. Binding experiments indicated that the specific binding is due to a soluble form of SV2A. In order to detect the specificity of the SV2A binding, the ability of levetiracetam and ucb 30889 to specifically bind to the soluble SV2A was examined. The affinities of the molecules were equivalent to that exhibited by ligands to the native membrane (Figure 21B). Scatchard analysis indicates that the K_D for [^{3}H]ucb 30889 for to the SV2A in native membrane is 30nM, while that for the soluble protein is 82 nM (Figure 21C). Thus, the binding properties of the soluble SV2A are similar to the membrane-bound native form indicative that the soluble protein maintains its native structural conformation in n-dodecyl-β-D-maltoside.

Affinity Purification of the Soluble SV2A and Identification of Putative SV2A Partners

Supernatants from solubilized membranes were incubated with anti-SV2A antibodies overnight at 4°C. The mixture was rotated with protein A-Sepharose beads for 1 hour at 4°C in Tris-HCl 20 mM pH 7.4, 0.25 M sucrose, protease inhibitors Complete (Roche). The resin was washed several times and the collected fractions contained immunopurified SV2A (Figure 22). As explained above, SV2A is maintained in its native conformation after solubilization in n-dodecyl-β-D-maltoside. Therefore, since synaptotagmin is a well known partner of SV2A, the immunopurified fractions were tested to determine whether synaptotagmin was still associated to SV2A after the purification procedure. Western analysis of the immunopurified fractions confirmed the presence of synaptotagmin associated to soluble SV2A, while the isoform SV2B was not detected. Thus, the solubilization and purification procedure can be used to perform SV2A-protein interactions studies.

Although the present invention has been described in detail with reference to examples above, it is understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims. All cited patents, patent applications and publications referred to in this application are herein incorporated by reference in their entirety.

WHAT IS CLAIMED IS:

1. A method of treating a neurological disorder associated with synaptic vesicle function, endocrinopathy or hormonal diseases, comprising administering a compound or agent that modulates a function or activity of an SV2 protein.

2. A method of claim 1, wherein the neurological disorder is selected from the group consisting of seizure, epilepsy, Parkinson's disease, Parkinson's dyskinesias, migraine, Alzheimer's disease, neuropathic pain, essential tremor, cognitive disorders, and movement disorders.

3. A method of claim 1, wherein the compound or agent binds to the levetiracetam binding site of an SV2 protein.

4. A method of modulating at least one function or activity of a SV2 protein in a cell, comprising exposing the cell to a compound or agent that binds to the levetiracetam binding site of the SV2 protein.

5. A method of claim 4, wherein the compound or agent modulates the binding of levetiracetam to the levetiracetam binding site.

6. A method of discovering or modeling an interaction between an SV2 protein and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site comprising:

a) contacting the SV2 protein with the compound or agent; and

b) measuring and analyzing the interaction of the SV2 protein with the compound or agent.

7. A method of claim 6 where the analysis is by proteolytic treatment of the SV2 proteins to observe a differential effect of binding of a ligand on proteolytic degradation.

8. A method of claim 6, wherein the analysis is by 3-dimensional modeling or other purely computational techniques.

9. A method of claim 8, wherein the 3-dimensional modeling is via nuclear magnetic resonance spectroscopy or X-ray crystallography.

10. A method of claim 6, wherein the analysis is by binding studies.

11. A method of any one of claims 6 or 10, wherein the SV2 protein is purified from natural sources

12. A method of claim 11 where the SV2 protein is purified from heterologously expressed protein driven from a cloned nucleotide inserted in an expression vector, in a eukaryotic or prokaryotic host.

13. A method of identifying a levetiracetam binding site within an SV2 protein comprising;

a) contacting a SV2 protein or fragment thereof with a compound or agent selected from the group consisting of levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site; and

b) determining the binding of the compound or agent with the SV2 protein or fragment thereof.

14. A method of claim 13, wherein the SV2 protein or fragment thereof comprises at least one amino acid substitution, deletion or addition.

15. A method of claim 14, wherein the addition, deletion or substitution of amino acid residues removes at least one glycosylation sites.

16. A method of claim 15, wherein the removal of glycosylation sites is *via* site-directed mutagenesis.

17. A method of claim 13, wherein the SV2 protein is a fusion protein comprising at least one SV2 protein or fragment thereof and a fusion partner.

18. A method of claim 17, wherein the fusion partner is a fusion tag.

19. A method of claim 18, wherein the fusion tag is a poly-His tag or glutathione-S-transferase.

20. A method of assaying the interaction between SV2 protein and a second protein comprising;

a) expressing SV2 protein and the protein of interest in a cell;

b) exposing the cell to a compound or agent which binds to the levetiracetam binding site; and

c) determining the interaction between the SV2 protein and the protein of interest.

21 A method of claim 20, wherein the second protein is selected from the group consisting of: a cell membrane protein, a vesicle membrane protein, a cytoplasmic protein, a cytoskeletal protein, and an intracellular matrix protein.

22. A method of claim 20, wherein the protein of interest is synaptotagmin.

23. A method of claim 20, wherein the protein of interest is a member of the SNARE complex.

24. A method of claim 23, wherein the member of the SNARE complex is synaptic vesicle associated VAMP/synaptobrevin, syntaxin, or SNAP-25.

25. A method of claim 20, wherein the SV2 protein lacks at least one glycosylation site.

26. A method of identifying a compound or agent that modulates a neurological disorder associated with synaptic function, endocrinopathy or hormonal disease comprising;

a) exposing a SV2 protein to the compound or agent; and

b) determining whether the compound or agent modulates an activity of the SV2 protein.

27. A method of any one of claim 3, 4, 5, 6, 20, or 26, wherein the compound or agent is levetiracetam or an analog or derivative thereof , or an anti-SV2 antibody or fragment thereof.

28. A method of any one of claims 13 or 27, wherein the compound or agent competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site.

29. A method of any one of claims 13 or 27, wherein the compound or agent is an anti-SV2 antibody or fragment thereof.

30. A method of claim 29, wherein the anti-SV2 antibody or fragment thereof binds to the levetiracetam binding site of SV2 protein.

31. A method of claim 29, wherein the anti-SV2 antibody or fragment thereof is selected from the group consisting of a polyclonal antibody and a monoclonal antibody.

32. A method of claim 31, wherein the antibody fragment is selected from the group consisting of an Fab fragment, Fab' fragment, $F(ab')_2$ fragment and an scFv fragment.

33. A method of claim 31, wherein the monoclonal antibody is selected from the group consisting of a chimeric antibody, a humanized antibody, and a human antibody.

34. A method of identifying a cellular response to a compound or agent selected from the group consisting of levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site comprising:

a) exposing cells expressing an SV2 protein to the compound or agent; and

b) analyzing a change in the expression of a nucleic acid or protein in the exposed cell.

35. A method of any one of claims 20 or 34, wherein the step of exposing the cell to a compound or agent which binds to the levetiracetam binding site is carried out under conditions with a divalent cation concentration selected from the group consisting of less than about 1 μM, between about 1 μM and about 1000 μM, and at least about 1000 μM.

36. An isolated nucleic acid molecule comprising the nucleic acid sequence of SEQ ID NO: 5 or the complement thereof.

37. An isolated polypeptide comprising an amino acid sequence encoded by the isolated nucleic acid molecule of claim 123.

38. An isolated polypeptide of claim 124, comprising the amino acid sequence of SEQ ID NO: 6.

39. A method of claim 26, wherein the step of determining whether the compound or agent modulates an activity of the SV2 protein is selected from the group consisting of

a) measuring transport of at least one monovalent cation or divalent cation across a membrane;

b) measuring SNARE complex formation;

c) measuring Ca^{2+} channel formation or activity;

d) measuring SV2 interaction with at least one other protein;

e) measuring transport of at least one substrate across a membrane; and,

f) measuring synaptic vesicle fusion, exocytosis, or synaptic vesicle recycling.

40. A method of claim 39, wherein the monovalent cation is selected from the group consisting of H^+, Cl^-, Na^+ and K^+.

41. A method of any one of claim 35 or 39, wherein the divalent cation is selected from the group consisting of Ca^{2+}, Zn^{2+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Cu^{2+}.

42. A method of claim 41, wherein the at least one divalent cation is Ca^{2+}.

43. A method of claim 39, wherein the at least one other protein is synaptotagmin.

44. A method of claim 39, wherein the at least one other protein is laminin-1.

45. A method of claim 39 wherein the at least one substrate is selected from the group consisting of amines, acetylcholine, excitatory neurotransmitters, GABA, serotonin, glycine or other amino acids, sugars and organic ions.

46. A method of identifying a binding partner for a SV2 protein, comprising:

a) exposing a SV2 protein or fragment to a potential binding partner;

b) incubating the protein or fragment and potential binding partner with (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide; and

c) determining if the binding of (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide to the protein is inhibited by the potential binding partner, thereby identifying binding partner for the protein.

47. A method of identifying a compound or agent useful for the treatment of a neurological or endocrinological disorder, comprising:

a) exposing a SV2 protein or fragment to the agent and levetiracetam or an analog or derivative thereof; and

b) determining if the binding of levetiracetam or an analog or derivative thereof to the protein is modulated by the agent, thereby identifying an agent useful for the treatment of a neurological disorder.

48. A method of claim 47, wherein the levetiracetam or an analog or derivative thereof is directly or indirectly labeled.

49. A method of claim 47, wherein the SV2 protein or fragment is incubated with the levetiracetam or an analog or derivative prior to the agent, after addition of the agent, or concurrent with the agent.

50. A method of claim 47, wherein the SV2 protein or fragment is incubated with levetiracetam.

51. A method of claim 47, wherein the neurological disorder is selected from the group consisting of epilepsy; epileptogenesis; seizure disorders; convulsions; withdrawal seizures; neurological disorders; bipolar disorders; mania; depression; anxiety; migraine; neuralgia; trigeminal neuralgia; chronic pain conditions; neuropathic pain; anaesthesia-related hyperexcitability; cerebral ischemia; head trauma; myotonia; exitatory states provoked by drug or alcohol abuse, dependence or withdrawal; stroke; myoclonus; essential tremor; tics; Tourette's syndrome; dyskinesia; spasticity; movement disorders; neonatal cerebral haemorrhage; amyotrophic lateral sclerosis; Parkinson's disease; Alzheimer's disease; a neurodegenerative disease; and dementia.

52. A pharmaceutical composition comprising a compound or agent as identified in the method of any one of claims 26 or 47 said compound being different from a compound as described in Fig. 15.

53. A method of treating a neurological or endocrinological disorder which comprises administering to an individual in need of such treatment a compound or agent as identified in the method of any one of claims 26 or 47 said compound being different from a compound as

described in Fig. 15.

54. A method according to claim 53, wherein the neurological disorder is selected from the group consisting of epilepsy; epileptogenesis; seizure disorders; convulsions; withdrawal seizures; neurological disorders; bipolar disorders; mania; depression; anxiety; migraine; neuralgia; trigeminal neuralgia; chronic pain conditions; neuropathic pain; anaesthesia-related hyperexcitability; cerebral ischemia; head trauma; myotonia; exitatory states provoked by drug or alcohol abuse, dependence or withdrawal; stroke; myoclonus; essential tremor; tics; Tourette's syndrome; dyskinesia; spasticity; movement disorders; neonatal cerebral haemorrhage; amyotrophic lateral sclerosis; Parkinson's disease; Alzheimer's disease; a neurodegenerative disease; and dementia.

55. A method according to claim 53 wherein the endocrinological disorders is selected from the group consisting of endocrinopathies involving hypersecretion or hyposecretion of at least one hormone; giantism; dwarfism; adrenal-medulla-related diseases; hypoglycemia; and circulation shock.

56. A method of any one of claims 1, 20, 26, 34, or 47, wherein the SV2 protein is SV2A.

57. A method of claim 56, wherein the SV2A protein comprises SEQ ID NO: 2.

58. A method of claim any one of claims 13, 27, or 47, wherein the analog or derivative of levetiracetam is (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide.

59. A method of claim 58, wherein the analog or derivative of levetiracetam is selected from the group consisting of N-alkylated 2-oxo-pyrrolidine derivatives, N-alkylated 2-oxo-piperidinyl derivatives, and N-alkylated 2-oxo-azepanyl derivatives.

60. A method of identifying an agent useful for the treatment of a neurological or endocrinological disorder, comprising:

a) exposing a SV2 protein or fragment to the agent;

b) incubating the protein or fragment and agent with (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide; and

c) determining if the binding of (2S)-2-[4-(3-azidophenyl)-2-oxopyrrolidin-1-yl]butanamide to the protein is inhibited by the agent, thereby identifying binding partners for the protein.

61. A method of discovering or modeling an interaction between an SV2 protein, or fragment or derivative thereof, and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site comprising:

a) creating a 3-dimensional model of the SV2 protein, or fragments thereof, via either biochemical, biophysical, purely computational techniques, or some combination of these; and

b) creating 3-dimensional model of one or a collection of potential ligands that might potentially bind the SV2 protein.

62. A method of claim 61, further comprising using purely computational techniques to dock the 3-dimensional model of SV2 proteins with the 3-dimensional models of potential ligands.

63. A method of discovering or modeling an interaction between an SV2 protein and a compound or agent selected from the group consisting of: levetiracetam, an analog or derivative of levetiracetam, or a compound or agent which competes with levetiracetam or an analog or derivative thereof for binding to the levetiracetam binding site comprising:

a) determining a biochemical, pharmacological, organismal, cellular or molecular effect of a potential CNS active molecule in a genetically wild-type animal or in molecules, cells or tissues derived from such animals; and

b) comparing the measured effect of that compound in an equivalent study in a system with an SV2 protein knocked out or knocked down.

64. A method of isolating a functionally active membrane associated SV2 protein complex comprising:

a) solubilizing tissues comprising the SV2 protein with a detergent; and

b) isolating the SV2 protein complex.

65. A method of claim 64, wherein the method further comprises purifying the SV2 protein complex by immunoaffinity.

66. A method of claim 65, wherein the SV2 protein complex is further purified to obtain the SV2 protein.

67. A method of claim 64, wherein the detergent is n-dodecyl-β-D-maltoside or derivatives or analogs thereof.

68. A method of claim 64, wherein the tissues are brain membranes.

69. A method of claim 64, further comprising identifying the molecule or molecules complexed to the SV2 protein.

70. A method of any one of claims 64 to 69, wherein the SV2 protein is SV2A protein, SV2B protein, or SV2C protein.

71. A purified SV2 protein complex obtained by the method of claim 64.

72. A purified SV2 protein complex of claim 71, wherein the SV2 protein is SV2A protein, SV2B protein, or SV2C protein.

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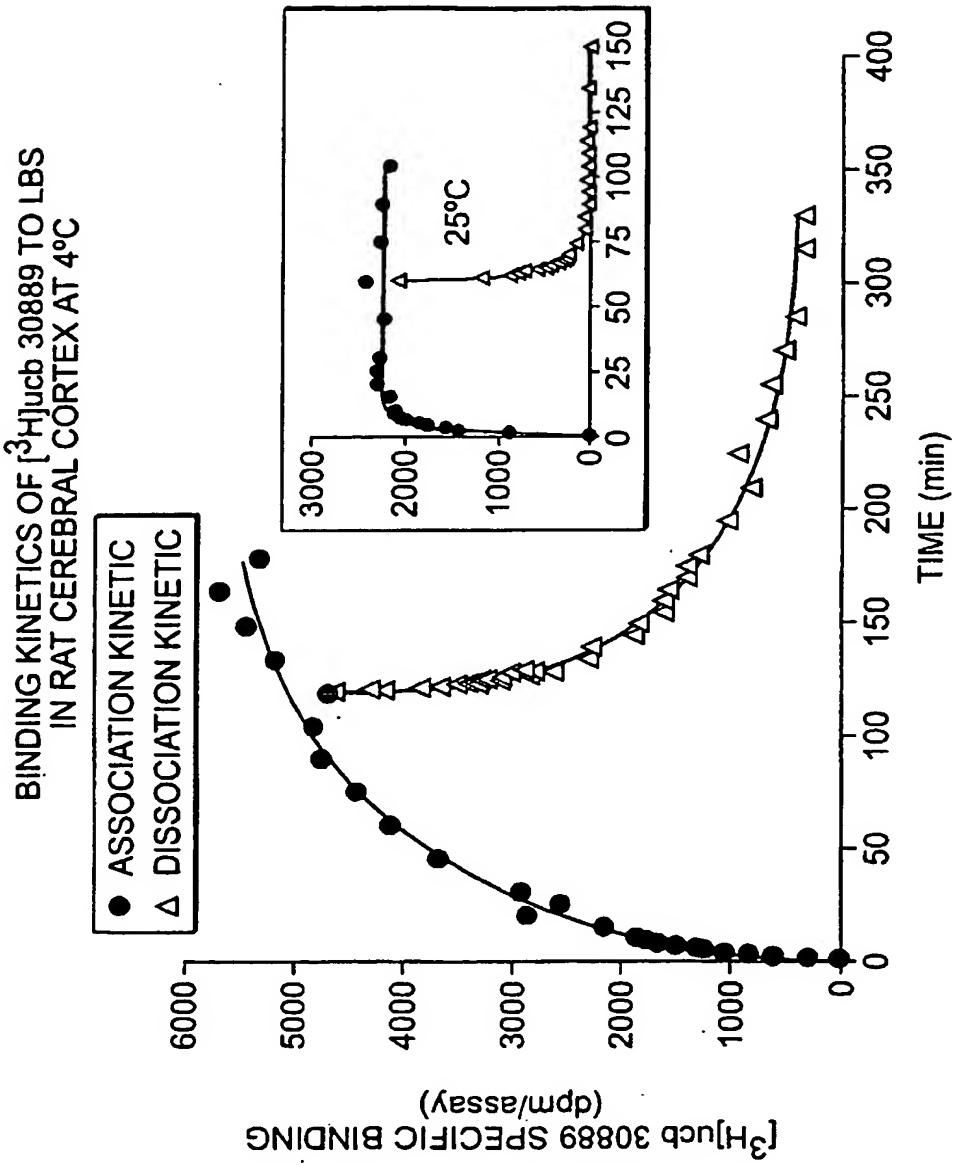

FIG. 1

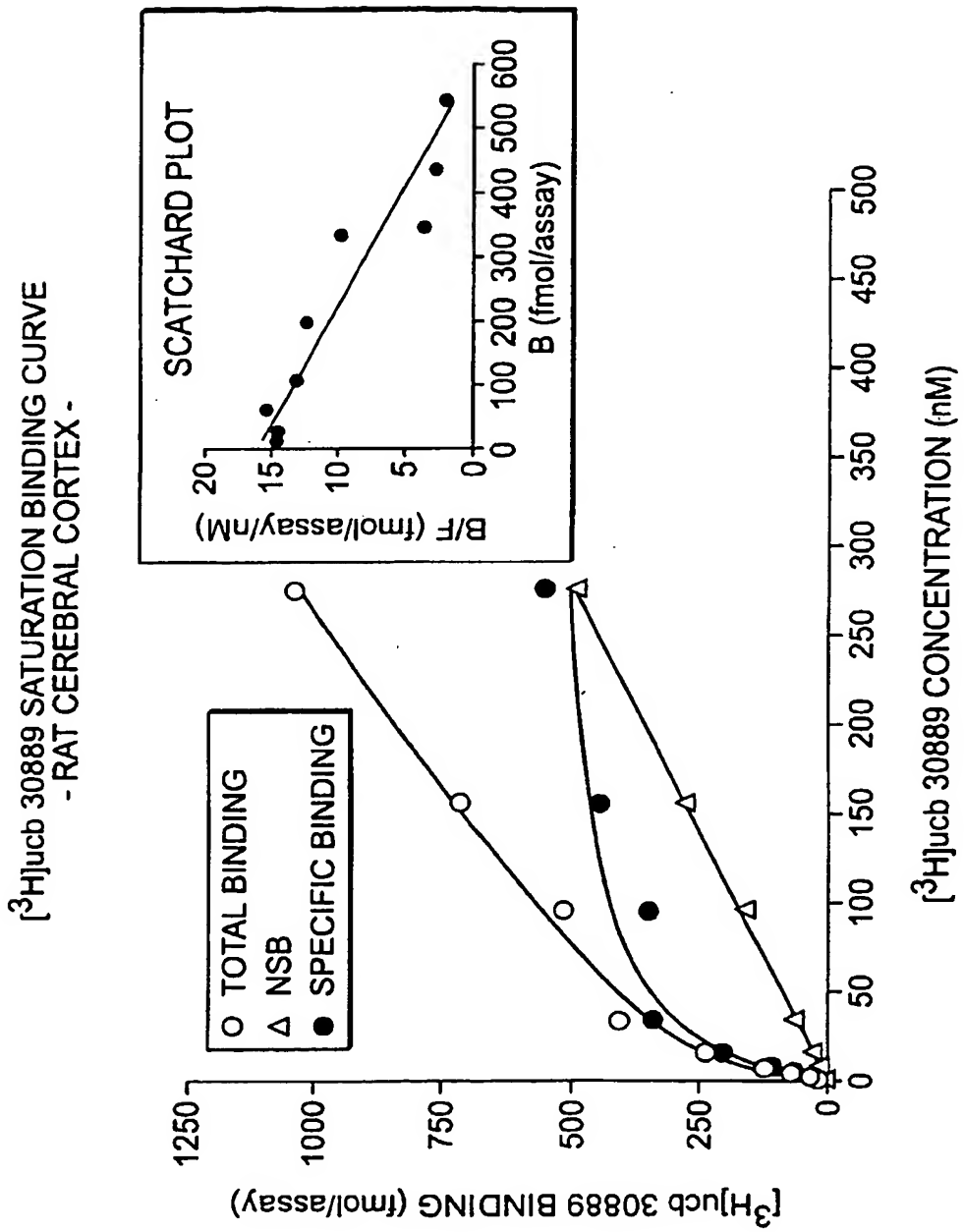
[3H]ucb 30889 SATURATION BINDING CURVE
- RAT CEREBRAL CORTEX -
O TOTAL BINDING
Δ NSB
● SPECIFIC BINDING
[3H]ucb 30889 BINDING (fmol/assay)
[3H]ucb 30889 CONCENTRATION (nM)
SCATCHARD PLOT
B/F (fmol/assay/nM)
B (fmol/assay)

FIG. 2

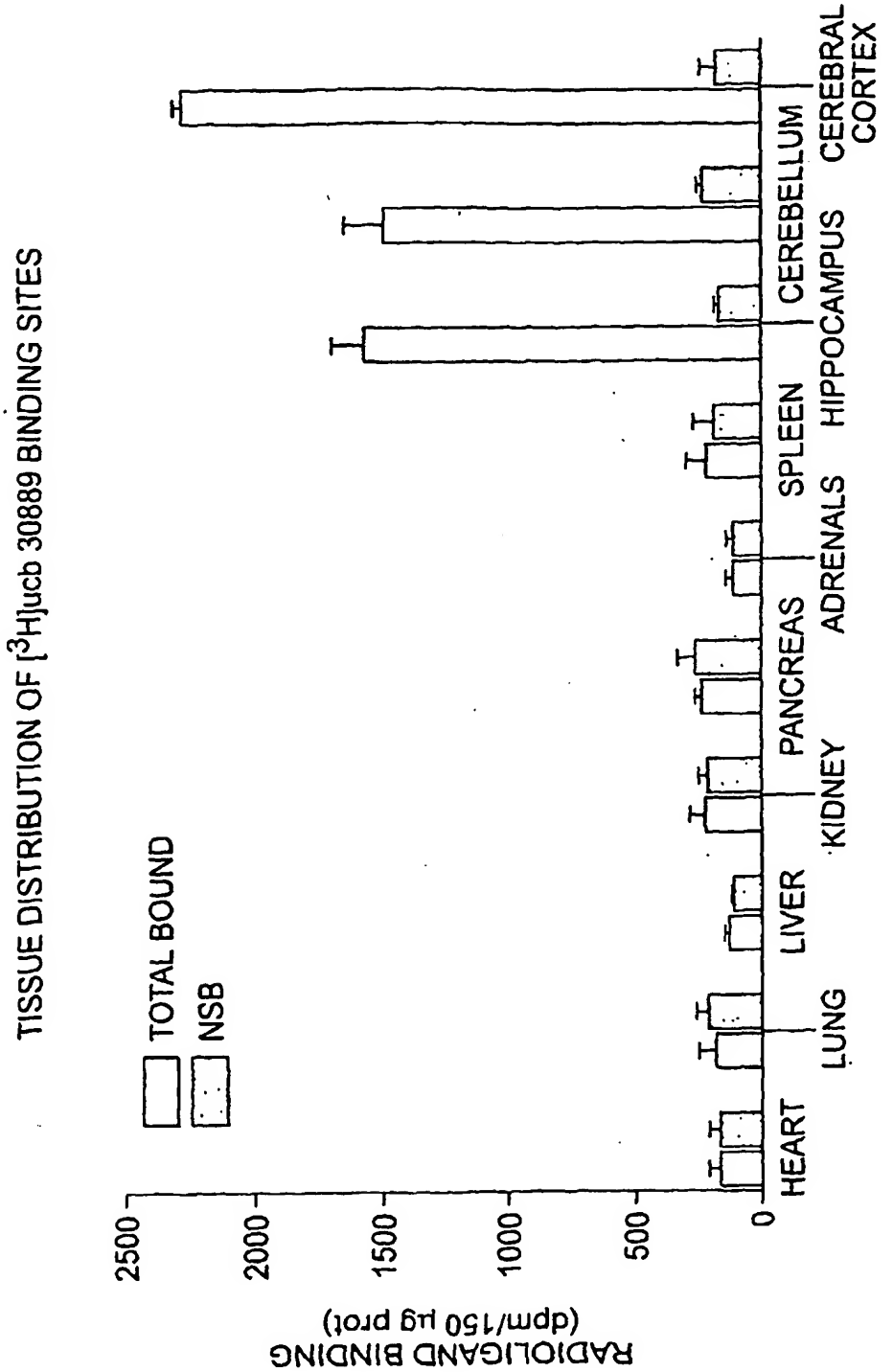
TISSUE DISTRIBUTION OF [3H]ucb 30889 BINDING SITES
TOTAL BOUND
NSB
RADIOLIGAND BINDING
(dpm/150 µg prot)
2500
2000
1500
1000
500
0
HEART
LUNG
LIVER
KIDNEY
PANCREAS
ADRENALS
SPLEEN
HIPPOCAMPUS
CEREBELLUM
CEREBRAL CORTEX

FIG. 3

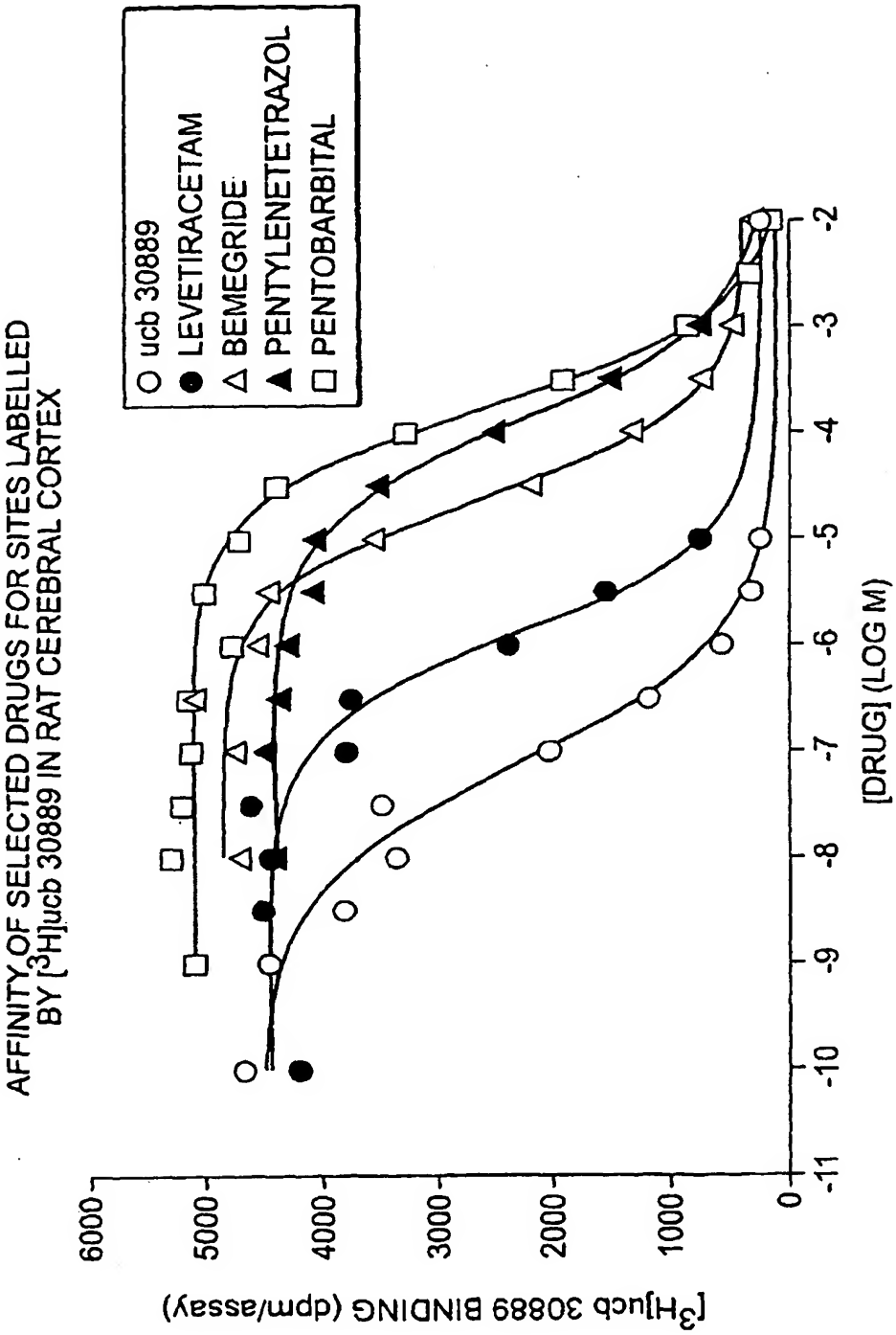
AFFINITY OF SELECTED DRUGS FOR SITES LABELLED BY [3H]ucb 30889 IN RAT CEREBRAL CORTEX
ucb 30889
LEVETIRACETAM
BEMEGRIDE
PENTYLENETETRAZOL
PENTOBARBITAL
[3H]ucb 30889 BINDING (dpm/assay)
6000
5000
4000
3000
2000
1000
0
-2
-3
-4
-5
-6
-7
-8
-9
-10
-11
[DRUG] (LOG M)

FIG. 4

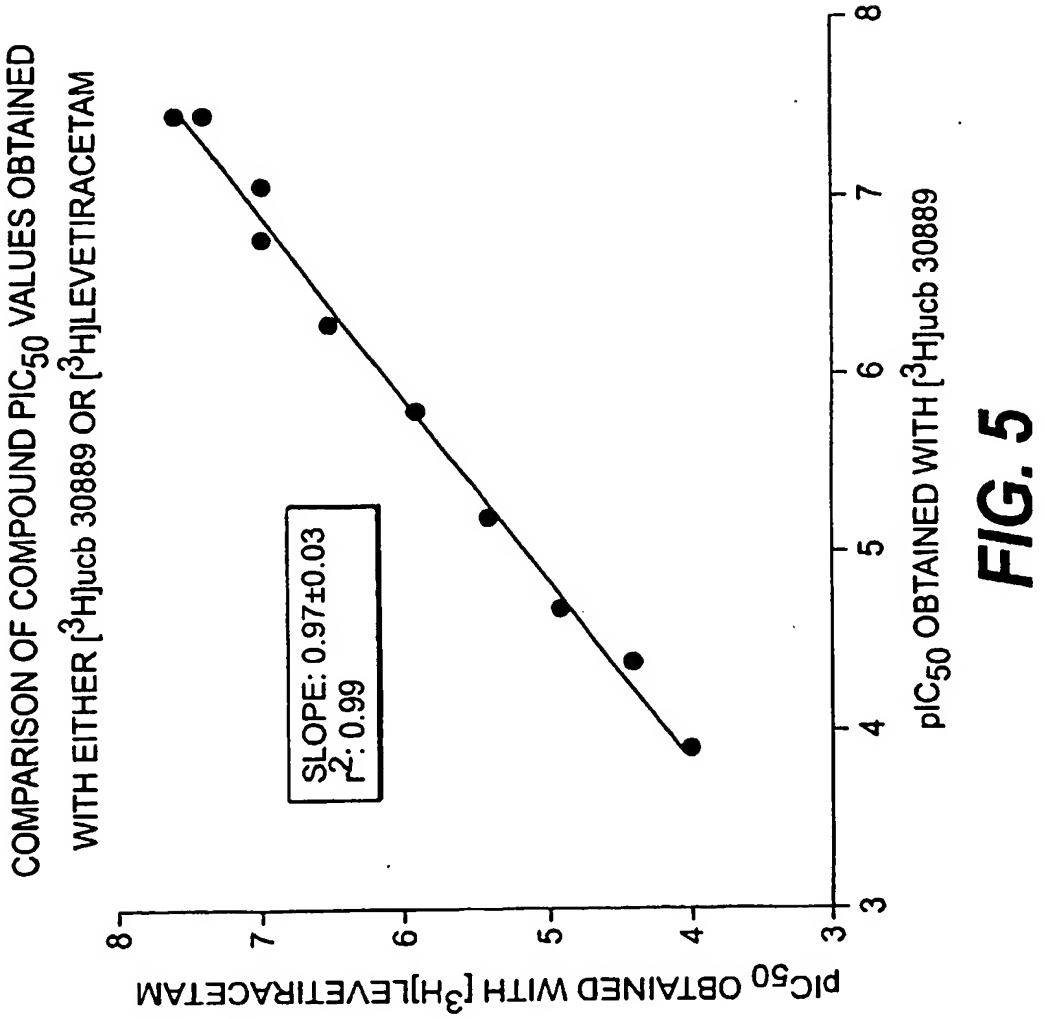
COMPARISON OF COMPOUND PIC50 VALUES OBTAINED WITH EITHER [3H]ucb 30889 OR [3H]LEVETIRACETAM
SLOPE: 0.97±0.03
r2: 0.99
pIC50 OBTAINED WITH [3H]LEVETIRACETAM
pIC50 OBTAINED WITH [3H]ucb 30889
3
4
5
6
7
8

FIG. 5

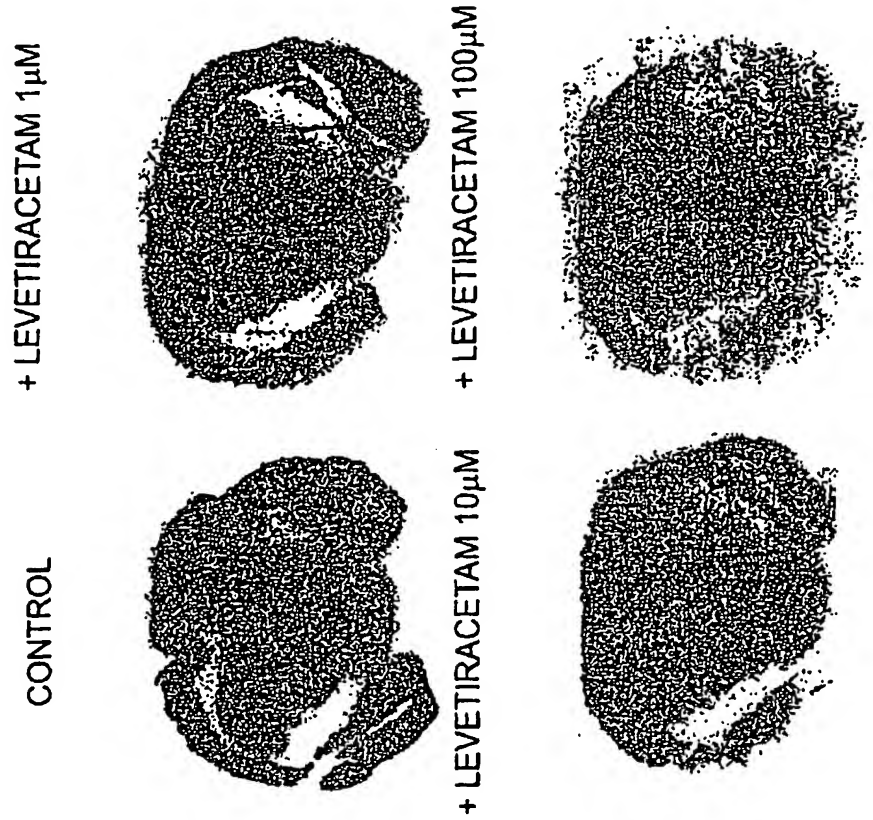

FIG. 6

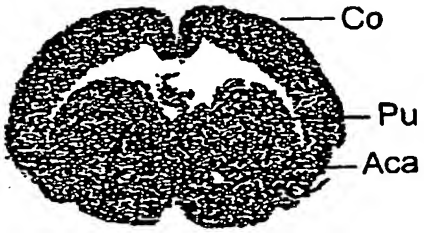

FIG. 7a

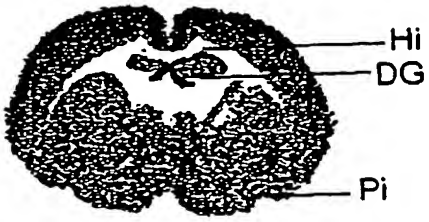

FIG. 7b

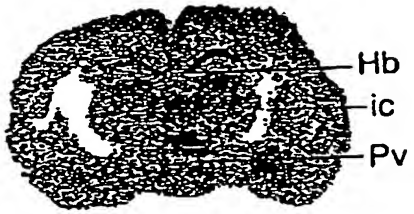

FIG. 7c

FIG. 7d

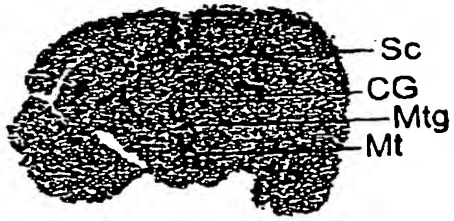

FIG. 7e

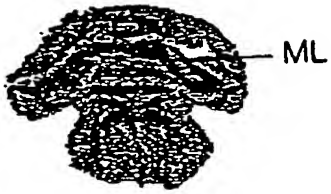

FIG. 7f

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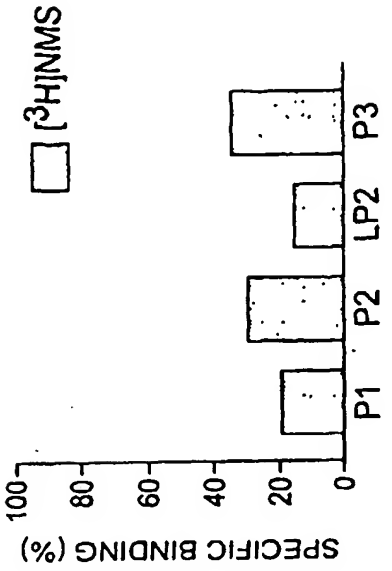


FIG. 8b

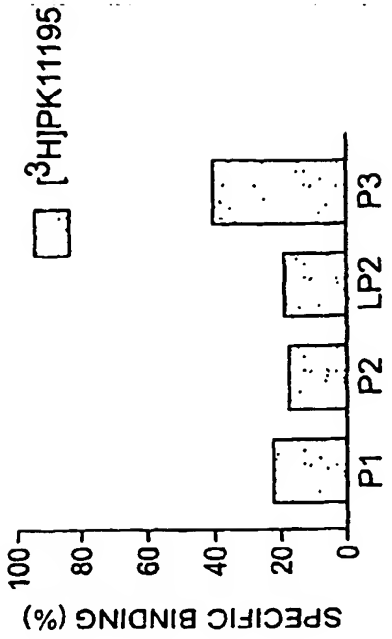


FIG. 8d

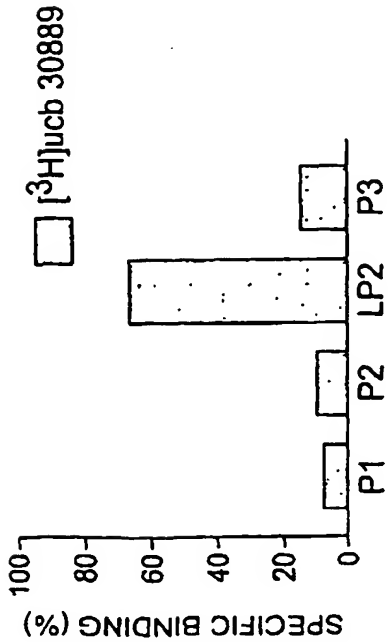


FIG. 8a

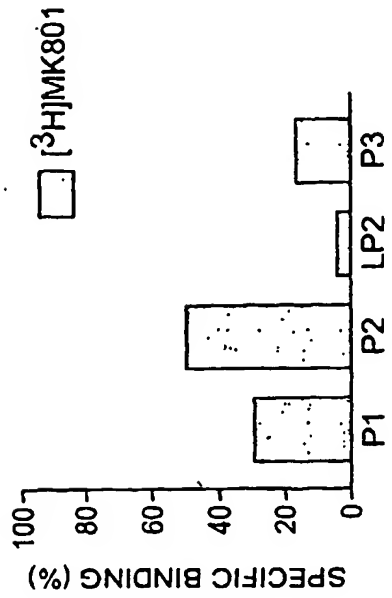


FIG. 8c

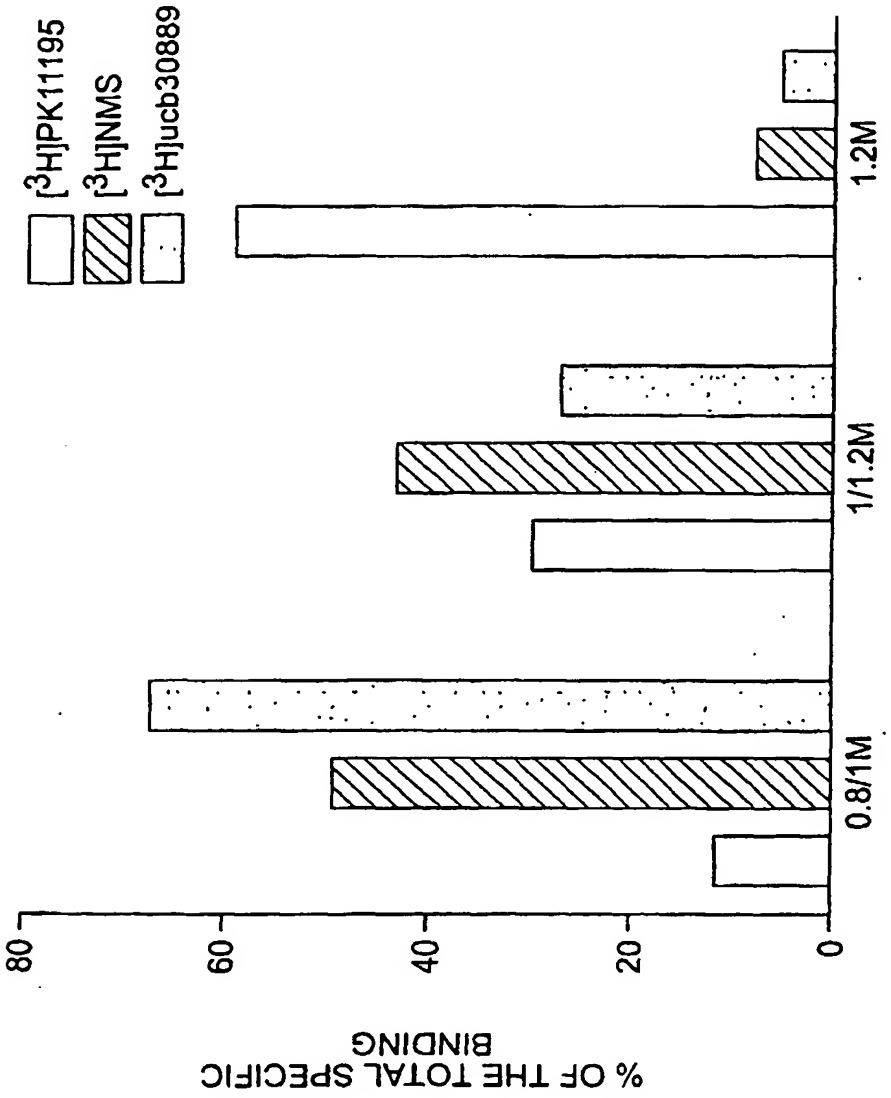

[3H]PK11195
[3H]NMS
[3H]ucb30889
1.2M
1/1.2M
0.8/1M
80
60
40
20
0
% OF THE TOTAL SPECIFIC BINDING

FIG. 9

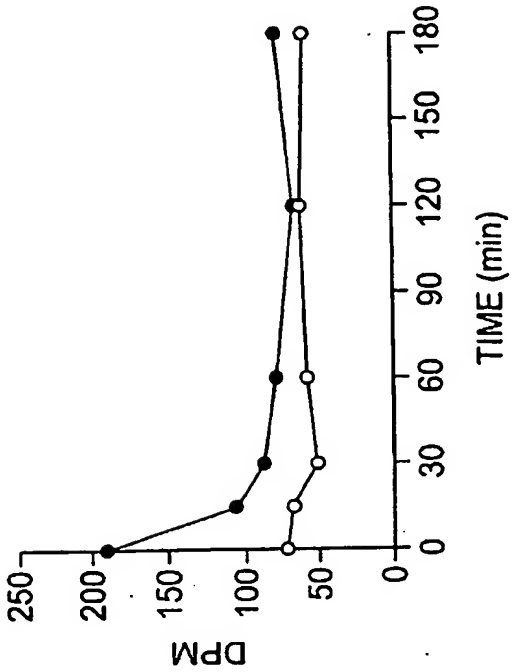


FIG. 10b

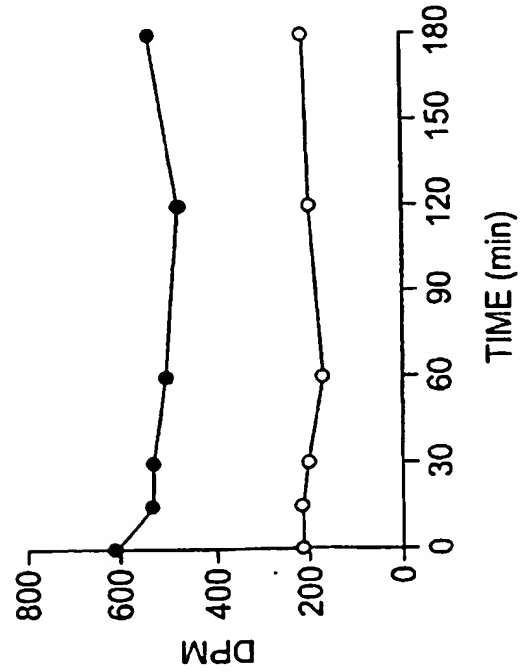


FIG. 10a

10/537512

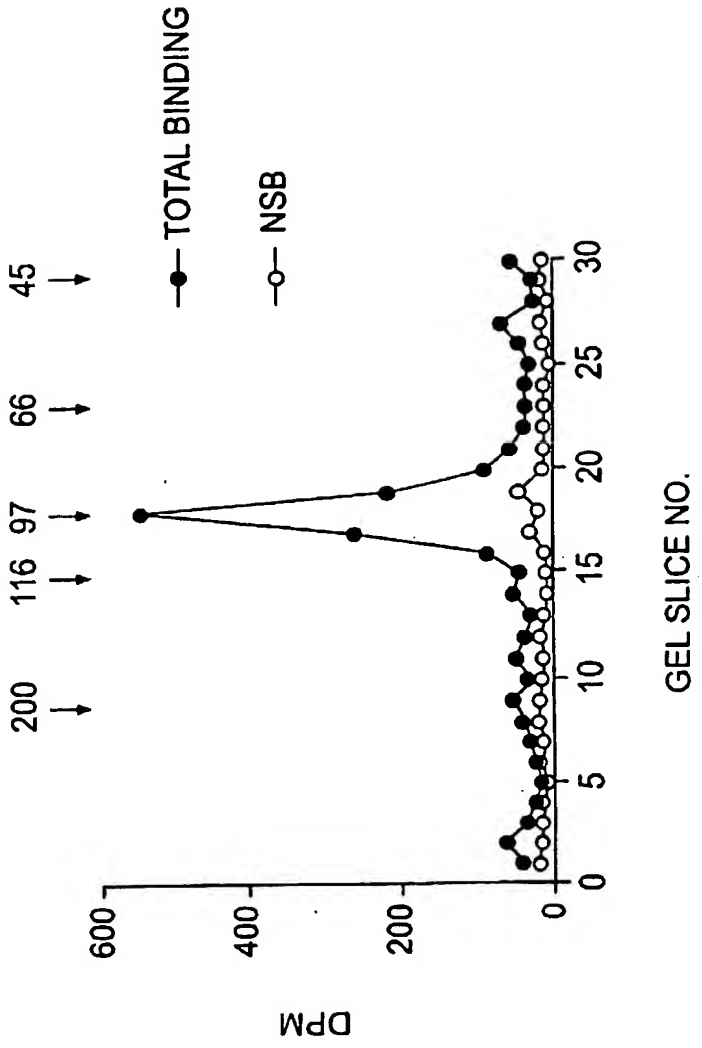


FIG. 11

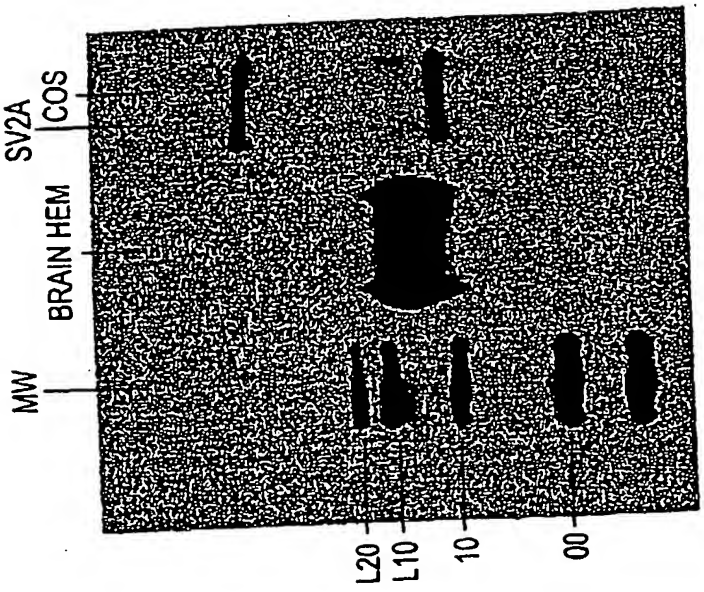


FIG. 12a

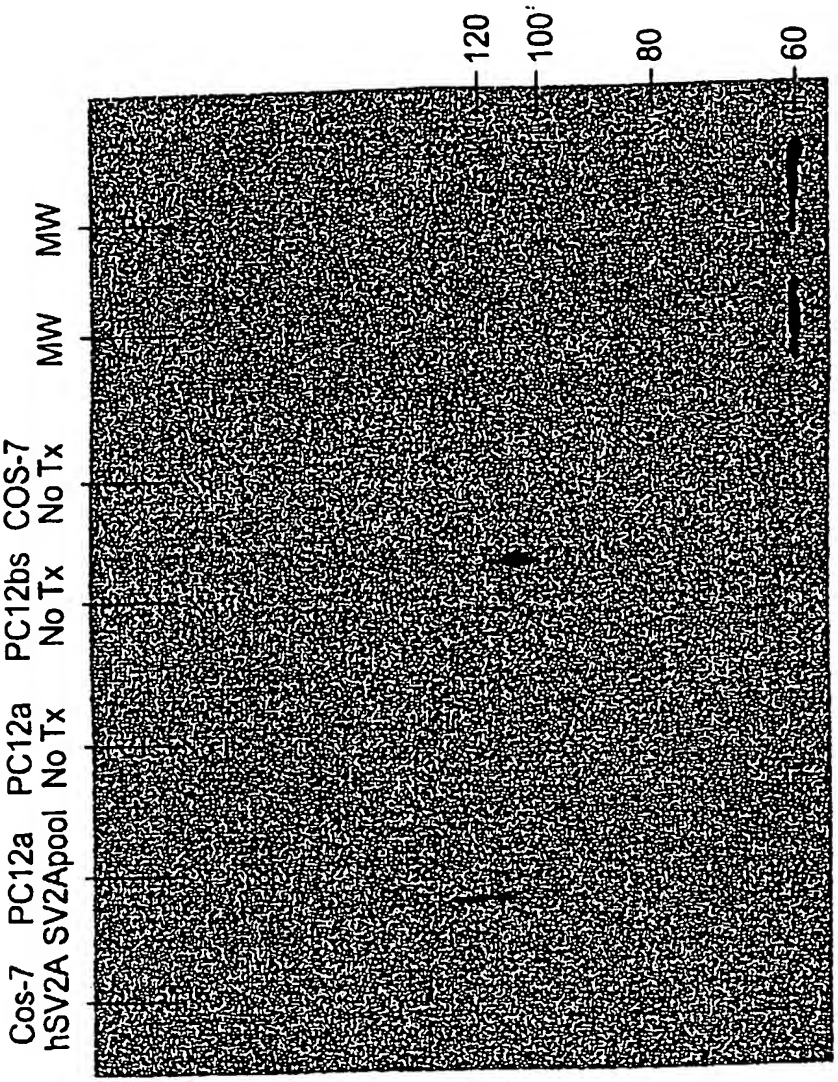

120
100
80
60
MW
MW
COS-7 No Tx
PC12bs No Tx
PC12a No Tx
PC12a SV2Apool
Cos-7 hSV2A

FIG. 12b

10/537512

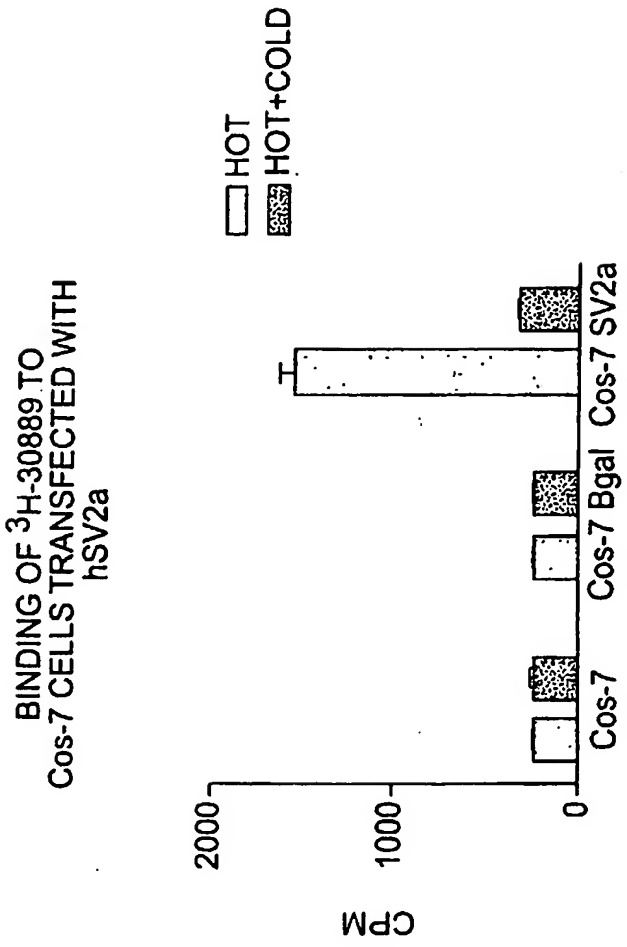


FIG. 13

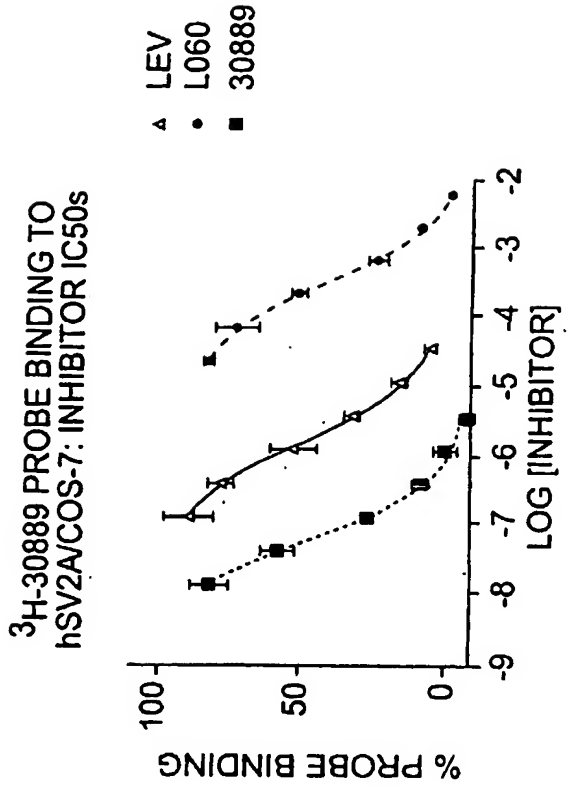
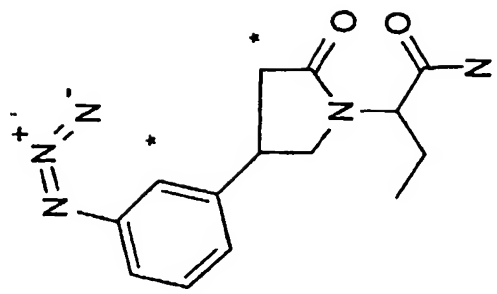

LEV
L060
30889
3H-30889 PROBE BINDING TO
hSV2A/COS-7: INHIBITOR IC50s
% PROBE BINDING
100
50
0
-2
-3
-4
-5
-6
-7
-8
-9
LOG [INHIBITOR]

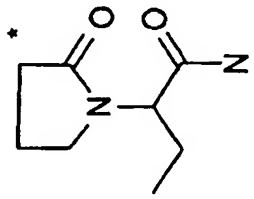
FIG. 14

10/537512



[^{3}H]ucb 30889

FIG. 15b



[^{3}H]LEVETIRACETAM

FIG. 15a

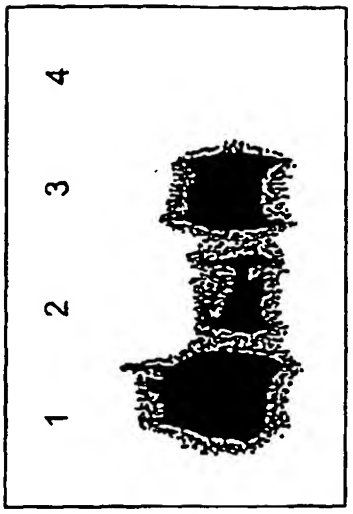


FIG. 16b

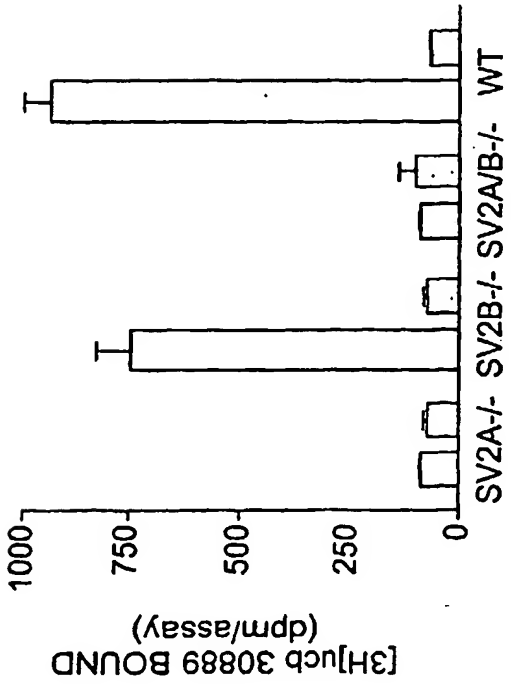


FIG. 16a

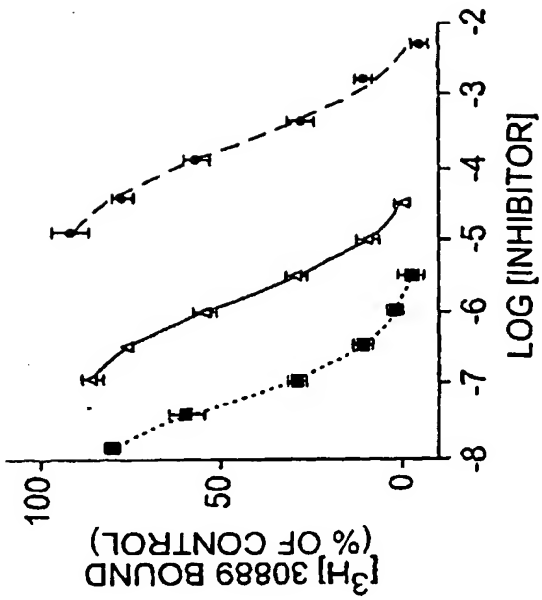


FIG. 17b

FIG. 17a

10/537512

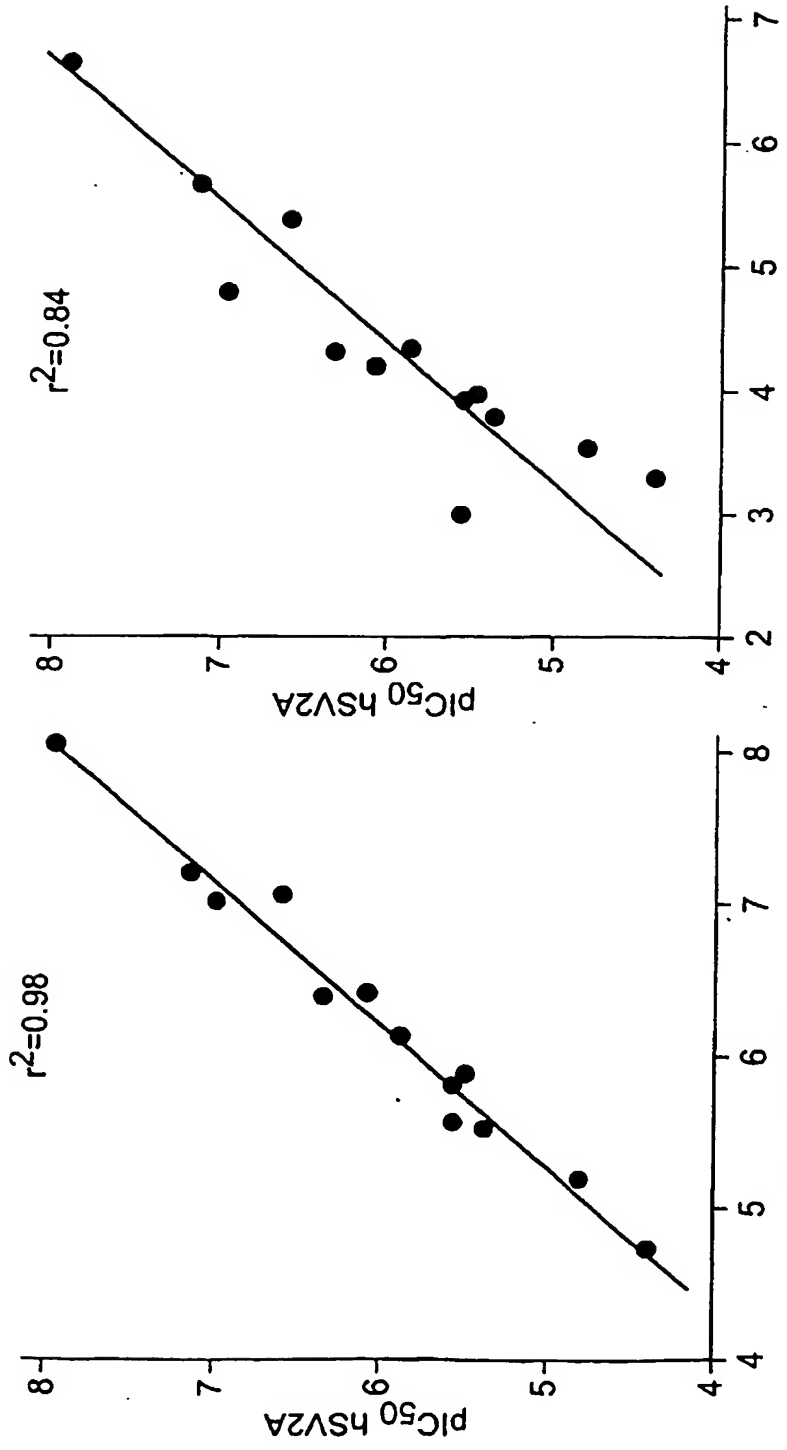


FIG. 18b

FIG. 18a

10/537512

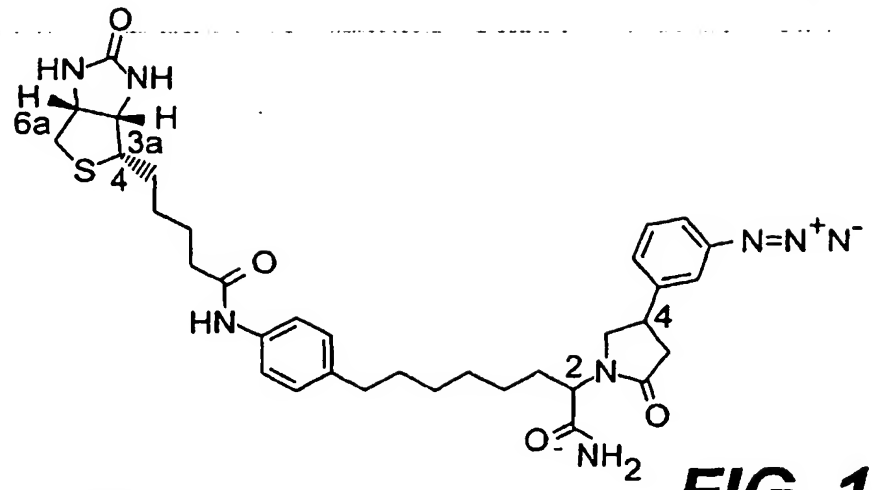
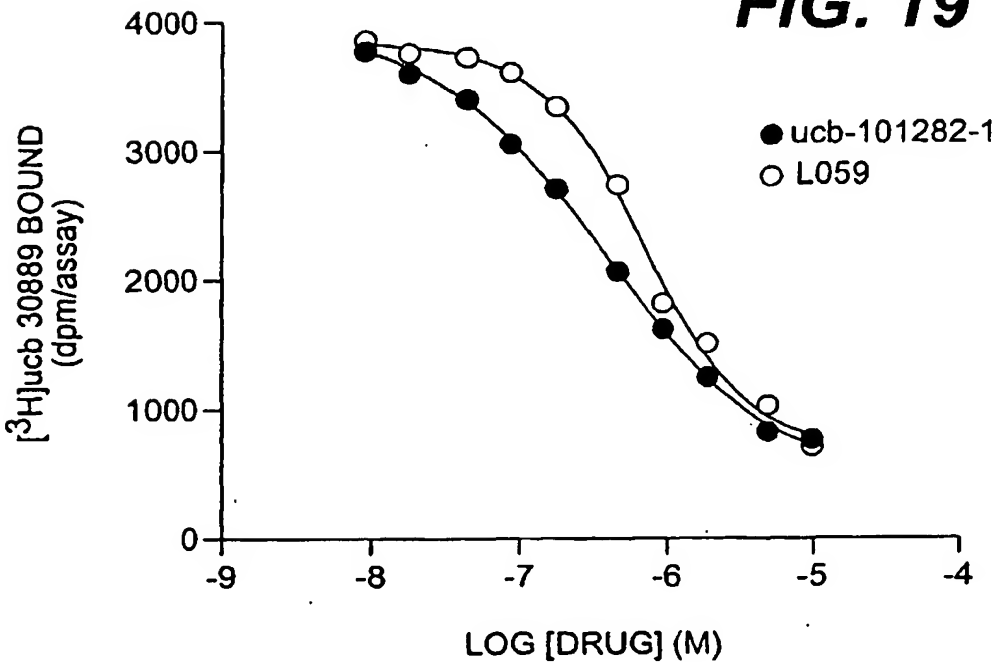


FIG. 19



	ucb-101282-1	L059
BOTTOM	458.4	661.8
TOP	3923	3836
LOGEC50	-6.392	-6.129
HILLSLOPE	-0.7946	-1.237
EC50	4.0510e-007	7.4320e-007

FIG. 20

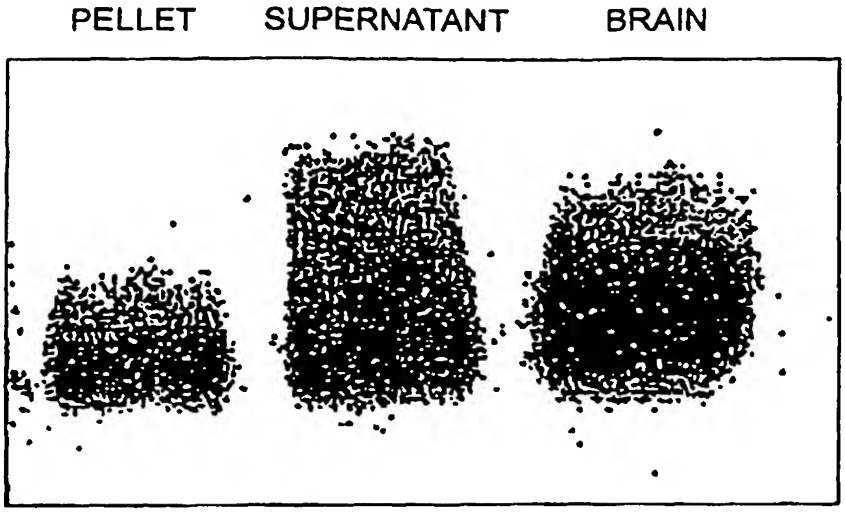
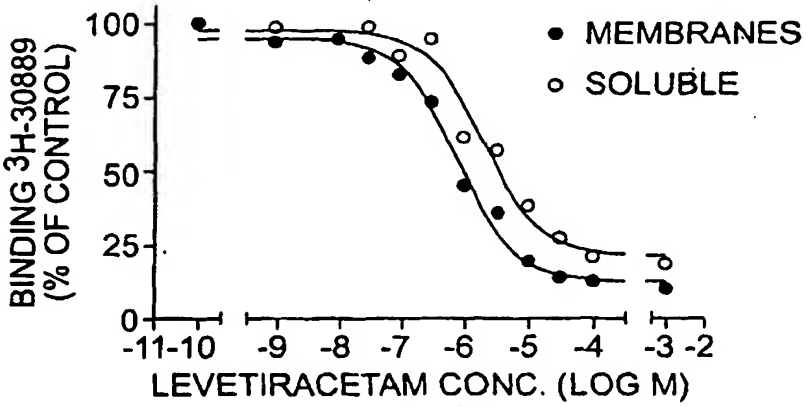


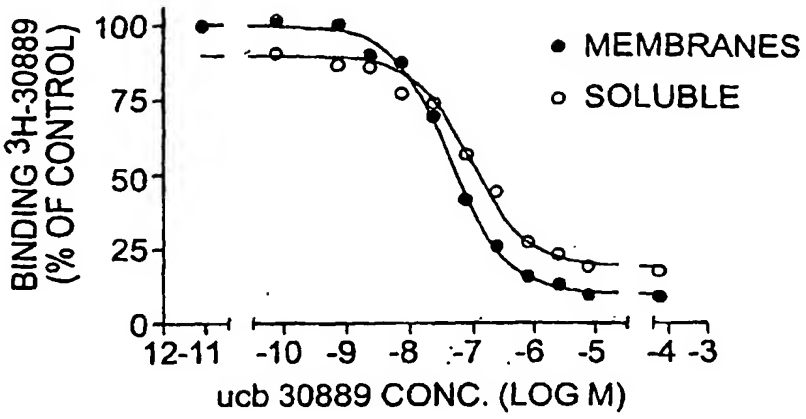
FIG. 21a



	MEMBRANES	SOLUBLE EXTRACTS
BOTTOM	94.97	97.71
TOP	12.30	21.03
LEGEC50	-6.104	-5.692
EC50	7.8770e-007	2.0340e-006

FIG. 21b

10/537512



	MEMBRANES	SOLUBLE EXTRACTS
BOTTOM	100.7	90.62
TOP	10.63	19.87
LEGEC50	-7.235	-6.922
EC50	5.8200e-008	1.1970e-007

FIG. 21c

SCATCHARD PLOT

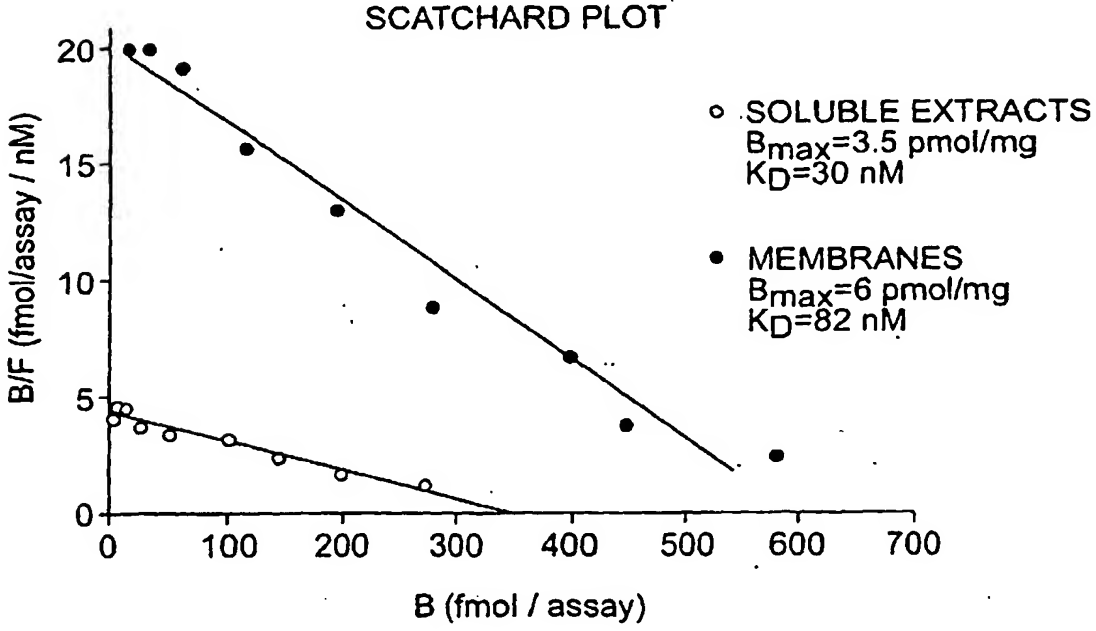


FIG. 22a

10/537512

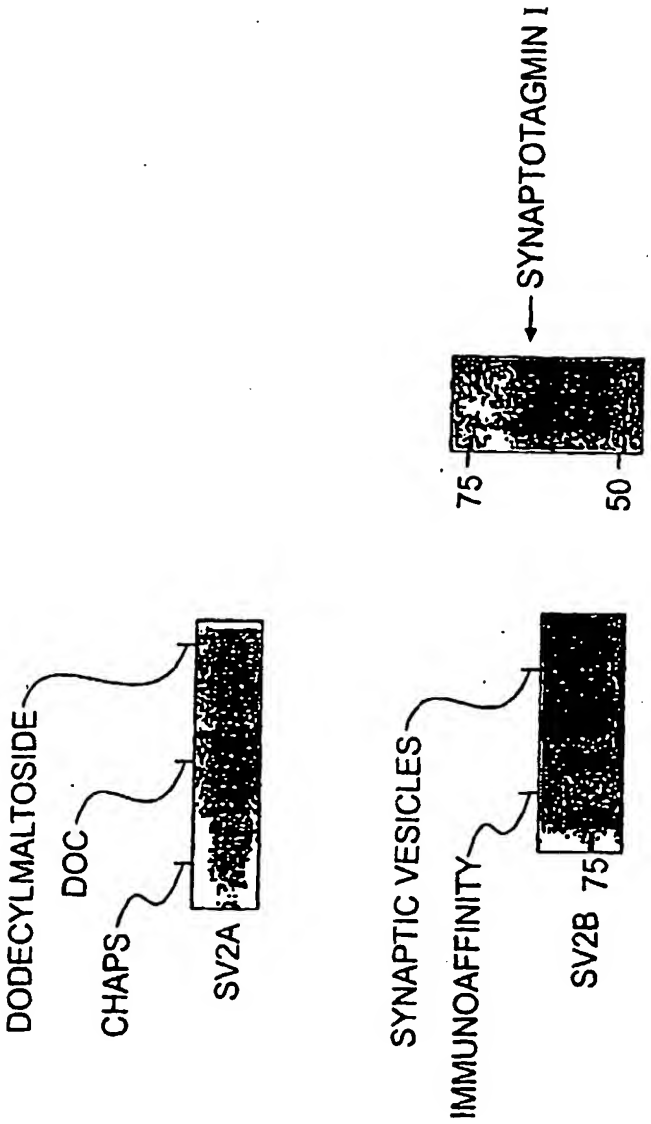


FIG. 22b

SEQUENCE LISTING

<110> UCB, S.A.
LYNCH, Berkley
NOCKA, Karl
FUKS, Bruno

<120> Methods for the identification of agents for the treatment of seizures, neurological diseases, endocrinopathies and hormonal diseases

<130> 53529-5007-01-WO

<150> US 60/430,372
<151> 2002-12-03

<150> US 60/506,764
<151> 2003-09-30

<160> 16

<170> PatentIn version 3.1

<210> 1
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<212> DNA
<213> Homo sapiens

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<223>

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Met Glu Glu Gly Phe Arg Asp Arg Ala Ala Phe Ile Arg Gly Ala Lys
1               5                   10                  15

gac att gct aag gaa gtc aaa aag cat gcg gcc aag aag gtg gtg aag       96
Asp Ile Ala Lys Glu Val Lys Lys His Ala Ala Lys Lys Val Val Lys
            20                  25                  30

ggc ctg gac aga gtc cag gac gaa tat tcc cga aga tcg tac tcc cgc      144
Gly Leu Asp Arg Val Gln Asp Glu Tyr Ser Arg Arg Ser Tyr Ser Arg
        35                  40                  45

ttt gag gag gag gat gat gat gat gac ttc cct gct ccc agt gat ggt      192
Phe Glu Glu Glu Asp Asp Asp Asp Asp Phe Pro Ala Pro Ser Asp Gly
    50                  55                  60

tat tac cga gga gaa ggg acc cag gat gag gag gaa ggt ggt gca tcc      240
Tyr Tyr Arg Gly Glu Gly Thr Gln Asp Glu Glu Glu Gly Gly Ala Ser
65                  70                  75                  80

agt gat gct act gag ggc cat gac gag gat gat gag atc tat gaa ggg      288
Ser Asp Ala Thr Glu Gly His Asp Glu Asp Asp Glu Ile Tyr Glu Gly
                85                  90                  95

gaa tat cag ggc att ccc cgg gca gag tct ggg ggc aaa ggc gag cgg      336
Glu Tyr Gln Gly Ile Pro Arg Ala Glu Ser Gly Gly Lys Gly Glu Arg
            100                 105                 110
```

```
atg gca gat ggg gcg ccc ctg gct gga gta agg ggg ggc ttg agt gat      384
Met Ala Asp Gly Ala Pro Leu Ala Gly Val Arg Gly Gly Leu Ser Asp
        115                 120                 125

ggg gag ggt ccc cct ggg ggc cgg ggg gag gca caa cga cgg aaa gaa      432
Gly Glu Gly Pro Pro Gly Gly Arg Gly Glu Ala Gln Arg Arg Lys Glu
    130                 135                 140

cga gaa gaa ctg gcc caa cag tat gaa gcc atc cta cgg gag tgt ggc      480
Arg Glu Glu Leu Ala Gln Gln Tyr Glu Ala Ile Leu Arg Glu Cys Gly
145                 150                 155                 160

cac ggc cgc ttc cag tgg aca ctg tat ttt gtg ctt ggt ctg gcg ctg      528
His Gly Arg Phe Gln Trp Thr Leu Tyr Phe Val Leu Gly Leu Ala Leu
                165                 170                 175

atg gct gac ggt gtg gag gtc ttt gtg gtg ggc ttc gtg ctg ccc agc      576
Met Ala Asp Gly Val Glu Val Phe Val Val Gly Phe Val Leu Pro Ser
            180                 185                 190

gct gag aaa gac atg tgc ctg tcc gac tcc aac aaa ggc atg cta ggc      624
Ala Glu Lys Asp Met Cys Leu Ser Asp Ser Asn Lys Gly Met Leu Gly
        195                 200                 205

ctc atc gtc tac ctg ggc atg atg gtg gga gcc ttc ctc tgg gga ggt      672
Leu Ile Val Tyr Leu Gly Met Met Val Gly Ala Phe Leu Trp Gly Gly
    210                 215                 220

ctg gct gac cgg ctg ggt cgg agg cag tgt ctg ctc atc tcg ctc tca      720
Leu Ala Asp Arg Leu Gly Arg Arg Gln Cys Leu Leu Ile Ser Leu Ser
225                 230                 235                 240

gtc aac agc gtc ttc gcc ttc ttc tca tct ttt gtc cag ggt tac ggc      768
Val Asn Ser Val Phe Ala Phe Phe Ser Ser Phe Val Gln Gly Tyr Gly
                245                 250                 255

act ttc ctc ttc tgc cgc cta ctt tct ggg gtt ggg att gga ggg tcc      816
Thr Phe Leu Phe Cys Arg Leu Leu Ser Gly Val Gly Ile Gly Gly Ser
            260                 265                 270

atc ccc att gtc ttc tcc tat ttc tcc gag ttt ctg gcc cag gag aaa      864
Ile Pro Ile Val Phe Ser Tyr Phe Ser Glu Phe Leu Ala Gln Glu Lys
        275                 280                 285

cga ggg gag cat ttg agc tgg ctc tgc atg ttt tgg atg att ggt ggc      912
Arg Gly Glu His Leu Ser Trp Leu Cys Met Phe Trp Met Ile Gly Gly
    290                 295                 300

gtg tac gca gct gct atg gcc tgg gcc atc atc ccc cac tat ggg tgg      960
Val Tyr Ala Ala Ala Met Ala Trp Ala Ile Ile Pro His Tyr Gly Trp
305                 310                 315                 320

agt ttt cag atg ggt tct gcc tac cag ttc cac agc tgg agg gtc ttc     1008
Ser Phe Gln Met Gly Ser Ala Tyr Gln Phe His Ser Trp Arg Val Phe
                325                 330                 335

gtc ctc gtc tgc gcc ttt cct tct gtg ttt gcc att ggg gct ctg acc     1056
Val Leu Val Cys Ala Phe Pro Ser Val Phe Ala Ile Gly Ala Leu Thr
            340                 345                 350
```

```
acg cag cct gag agc ccc cgt ttc ttc cta gag aat gga aag cat gat      1104
Thr Gln Pro Glu Ser Pro Arg Phe Phe Leu Glu Asn Gly Lys His Asp
        355                 360                 365

gag gcc tgg atg gtg ctg aag cag gtc cat gat acc aac atg cga gcc      1152
Glu Ala Trp Met Val Leu Lys Gln Val His Asp Thr Asn Met Arg Ala
    370                 375                 380

aaa gga cat cct gag cga gtg ttc tca gta acc cac att aag acg att      1200
Lys Gly His Pro Glu Arg Val Phe Ser Val Thr His Ile Lys Thr Ile
385                 390                 395                 400

cat cag gag gat gaa ttg att gag atc cag tcg gac aca ggg acc tgg      1248
His Gln Glu Asp Glu Leu Ile Glu Ile Gln Ser Asp Thr Gly Thr Trp
                405                 410                 415

tac cag cgc tgg ggg gtc cgg gcc ttg agc cta ggg ggg cag gtt tgg      1296
Tyr Gln Arg Trp Gly Val Arg Ala Leu Ser Leu Gly Gly Gln Val Trp
            420                 425                 430

ggg aat ttt ctc tcc tgt ttt ggt ccc gaa tat cgg cgc atc act ctg      1344
Gly Asn Phe Leu Ser Cys Phe Gly Pro Glu Tyr Arg Arg Ile Thr Leu
        435                 440                 445

atg atg atg ggt gtg tgg ttc acc atg tca ttc agc tac tat ggc ctg      1392
Met Met Met Gly Val Trp Phe Thr Met Ser Phe Ser Tyr Tyr Gly Leu
    450                 455                 460

acc gtc tgg ttt cct gac atg atc cgc cat ctc cag gca gtg gac tac      1440
Thr Val Trp Phe Pro Asp Met Ile Arg His Leu Gln Ala Val Asp Tyr
465                 470                 475                 480

gca tcc cgc acc aaa gtg ttc ccc ggg gag cgc gta gag cat gta act      1488
Ala Ser Arg Thr Lys Val Phe Pro Gly Glu Arg Val Glu His Val Thr
                485                 490                 495

ttt aac ttc acg ttg gag aat cag atc cac cga ggc ggg cag tac ttc      1536
Phe Asn Phe Thr Leu Glu Asn Gln Ile His Arg Gly Gly Gln Tyr Phe
            500                 505                 510

aat gac aag ttc att ggg ctg cgg ctc aag tca gtg tcc ttt gag gat      1584
Asn Asp Lys Phe Ile Gly Leu Arg Leu Lys Ser Val Ser Phe Glu Asp
        515                 520                 525

tcc ctg ttt gaa gag tgt tat ttt gag gat gtc aca tcc agc aac acg      1632
Ser Leu Phe Glu Glu Cys Tyr Phe Glu Asp Val Thr Ser Ser Asn Thr
    530                 535                 540

ttt ttc cgc aac tgc aca ttc atc aac act gtg ttc tat aac act gac      1680
Phe Phe Arg Asn Cys Thr Phe Ile Asn Thr Val Phe Tyr Asn Thr Asp
545                 550                 555                 560

ctg ttc gag tac aag ttt gtg aac agc cgt ctg ata aac agt aca ttc      1728
Leu Phe Glu Tyr Lys Phe Val Asn Ser Arg Leu Ile Asn Ser Thr Phe
                565                 570                 575

ctg cac aac aag gag ggc tgc ccg cta gac gtg aca ggg acg ggc gaa      1776
Leu His Asn Lys Glu Gly Cys Pro Leu Asp Val Thr Gly Thr Gly Glu
            580                 585                 590

ggt gcc tac atg gta tac ttt gtg agc ttc ctg ggg aca ctg gca gtg      1824
```

```
Gly Ala Tyr Met Val Tyr Phe Val Ser Phe Leu Gly Thr Leu Ala Val
        595                 600                 605

ctt cct ggg aat atc gtg tct gcc ctg ctc atg gac aag atc ggc agg      1872
Leu Pro Gly Asn Ile Val Ser Ala Leu Leu Met Asp Lys Ile Gly Arg
    610                 615                 620

ctc aga atg ctt gct ggc tcc agc gtg atg tcc tgt gtc tcc tgc ttc      1920
Leu Arg Met Leu Ala Gly Ser Ser Val Met Ser Cys Val Ser Cys Phe
625                 630                 635                 640

ttc ctg tct ttt ggg aac agt gag tcg gcc atg atc gct ctg ctc tgc      1968
Phe Leu Ser Phe Gly Asn Ser Glu Ser Ala Met Ile Ala Leu Leu Cys
                645                 650                 655

ctt ttt ggc ggg gtc agc att gca tcc tgg aat gcg ctg gac gtg ttg      2016
Leu Phe Gly Gly Val Ser Ile Ala Ser Trp Asn Ala Leu Asp Val Leu
            660                 665                 670

act gtt gaa ctc tac ccc tca gac aag agg acc aca gct ttt ggc ttc      2064
Thr Val Glu Leu Tyr Pro Ser Asp Lys Arg Thr Thr Ala Phe Gly Phe
        675                 680                 685

ctg aat gcc ctg tgt aag ctg gca gct gtg ctg ggg atc agc atc ttc      2112
Leu Asn Ala Leu Cys Lys Leu Ala Ala Val Leu Gly Ile Ser Ile Phe
    690                 695                 700

aca tcc ttc gtg gga atc acc aag gct gca ccc atc ctc ttt gcc tca      2160
Thr Ser Phe Val Gly Ile Thr Lys Ala Ala Pro Ile Leu Phe Ala Ser
705                 710                 715                 720

gct gcc ctt gcc ctt ggc agc tct ctg gcc ctg aag ctg cct gag acc      2208
Ala Ala Leu Ala Leu Gly Ser Ser Leu Ala Leu Lys Leu Pro Glu Thr
                725                 730                 735

cgg ggg cag gtg ctg cag tga                                          2229
Arg Gly Gln Val Leu Gln
            740
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<210> 2
<211> 742
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<213> Homo sapiens

<400> 2

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1               5                   10                  15

Asp Ile Ala Lys Glu Val Lys Lys His Ala Ala Lys Lys Val Val Lys
            20                  25                  30

Gly Leu Asp Arg Val Gln Asp Glu Tyr Ser Arg Arg Ser Tyr Ser Arg
        35                  40                  45

Phe Glu Glu Glu Asp Asp Asp Asp Asp Phe Pro Ala Pro Ser Asp Gly
    50                  55                  60

Tyr Tyr Arg Gly Glu Gly Thr Gln Asp Glu Glu Glu Gly Gly Ala Ser
65                  70                  75                  80
```

```
Ser Asp Ala Thr Glu Gly His Asp Glu Asp Asp Glu Ile Tyr Glu Gly
                85                  90                  95

Glu Tyr Gln Gly Ile Pro Arg Ala Glu Ser Gly Gly Lys Gly Glu Arg
            100                 105                 110

Met Ala Asp Gly Ala Pro Leu Ala Gly Val Arg Gly Gly Leu Ser Asp
        115                 120                 125

Gly Glu Gly Pro Pro Gly Gly Arg Gly Glu Ala Gln Arg Arg Lys Glu
    130                 135                 140

Arg Glu Glu Leu Ala Gln Gln Tyr Glu Ala Ile Leu Arg Glu Cys Gly
145                 150                 155                 160

His Gly Arg Phe Gln Trp Thr Leu Tyr Phe Val Leu Gly Leu Ala Leu
                165                 170                 175

Met Ala Asp Gly Val Glu Val Phe Val Val Gly Phe Val Leu Pro Ser
            180                 185                 190

Ala Glu Lys Asp Met Cys Leu Ser Asp Ser Asn Lys Gly Met Leu Gly
        195                 200                 205

Leu Ile Val Tyr Leu Gly Met Met Val Gly Ala Phe Leu Trp Gly Gly
    210                 215                 220

Leu Ala Asp Arg Leu Gly Arg Arg Gln Cys Leu Leu Ile Ser Leu Ser
225                 230                 235                 240

Val Asn Ser Val Phe Ala Phe Phe Ser Ser Phe Val Gln Gly Tyr Gly
                245                 250                 255

Thr Phe Leu Phe Cys Arg Leu Leu Ser Gly Val Gly Ile Gly Gly Ser
            260                 265                 270

Ile Pro Ile Val Phe Ser Tyr Phe Ser Glu Phe Leu Ala Gln Glu Lys
        275                 280                 285

Arg Gly Glu His Leu Ser Trp Leu Cys Met Phe Trp Met Ile Gly Gly
    290                 295                 300

Val Tyr Ala Ala Ala Met Ala Trp Ala Ile Ile Pro His Tyr Gly Trp
305                 310                 315                 320

Ser Phe Gln Met Gly Ser Ala Tyr Gln Phe His Ser Trp Arg Val Phe
                325                 330                 335

Val Leu Val Cys Ala Phe Pro Ser Val Phe Ala Ile Gly Ala Leu Thr
            340                 345                 350

Thr Gln Pro Glu Ser Pro Arg Phe Phe Leu Glu Asn Gly Lys His Asp
        355                 360                 365

Glu Ala Trp Met Val Leu Lys Gln Val His Asp Thr Asn Met Arg Ala
    370                 375                 380

Lys Gly His Pro Glu Arg Val Phe Ser Val Thr His Ile Lys Thr Ile
385                 390                 395                 400

His Gln Glu Asp Glu Leu Ile Glu Ile Gln Ser Asp Thr Gly Thr Trp
```

405 410 415

Tyr Gln Arg Trp Gly Val Arg Ala Leu Ser Leu Gly Gly Gln Val Trp
420 425 430

Gly Asn Phe Leu Ser Cys Phe Gly Pro Glu Tyr Arg Arg Ile Thr Leu
435 440 445

Met Met Met Gly Val Trp Phe Thr Met Ser Phe Ser Tyr Tyr Gly Leu
450 455 460

Thr Val Trp Phe Pro Asp Met Ile Arg His Leu Gln Ala Val Asp Tyr
465 470 475 480

Ala Ser Arg Thr Lys Val Phe Pro Gly Glu Arg Val Glu His Val Thr
485 490 495

Phe Asn Phe Thr Leu Glu Asn Gln Ile His Arg Gly Gly Gln Tyr Phe
500 505 510

Asn Asp Lys Phe Ile Gly Leu Arg Leu Lys Ser Val Ser Phe Glu Asp
515 520 525

Ser Leu Phe Glu Glu Cys Tyr Phe Glu Asp Val Thr Ser Ser Asn Thr
530 535 540

Phe Phe Arg Asn Cys Thr Phe Ile Asn Thr Val Phe Tyr Asn Thr Asp
545 550 555 560

Leu Phe Glu Tyr Lys Phe Val Asn Ser Arg Leu Ile Asn Ser Thr Phe
565 570 575

Leu His Asn Lys Glu Gly Cys Pro Leu Asp Val Thr Gly Thr Gly Glu
580 585 590

Gly Ala Tyr Met Val Tyr Phe Val Ser Phe Leu Gly Thr Leu Ala Val
595 600 605

Leu Pro Gly Asn Ile Val Ser Ala Leu Leu Met Asp Lys Ile Gly Arg
610 615 620

Leu Arg Met Leu Ala Gly Ser Ser Val Met Ser Cys Val Ser Cys Phe
625 630 635 640

Phe Leu Ser Phe Gly Asn Ser Glu Ser Ala Met Ile Ala Leu Leu Cys
645 650 655

Leu Phe Gly Gly Val Ser Ile Ala Ser Trp Asn Ala Leu Asp Val Leu
660 665 670

Thr Val Glu Leu Tyr Pro Ser Asp Lys Arg Thr Thr Ala Phe Gly Phe
675 680 685

Leu Asn Ala Leu Cys Lys Leu Ala Ala Val Leu Gly Ile Ser Ile Phe
690 695 700

Thr Ser Phe Val Gly Ile Thr Lys Ala Ala Pro Ile Leu Phe Ala Ser
705 710 715 720

Ala Ala Leu Ala Leu Gly Ser Ser Leu Ala Leu Lys Leu Pro Glu Thr
725 730 735

Arg Gly Gln Val Leu Gln
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Met Asp Asp Tyr Lys Tyr Gln Asp Asn Tyr Gly Gly Tyr Ala Pro Ser
1               5                  10                  15

gat ggc tat tac cgc ggc aat gag tcc aac cca gaa gaa gat gca cag      96
Asp Gly Tyr Tyr Arg Gly Asn Glu Ser Asn Pro Glu Glu Asp Ala Gln
            20                  25                  30

agt gat gtc acc gaa ggc cat gat gag gaa gac gag atc tat gag ggc     144
Ser Asp Val Thr Glu Gly His Asp Glu Glu Asp Glu Ile Tyr Glu Gly
        35                  40                  45

gag tac cag ggt atc cct cac cca gat gat gtc aag gcc aag cag gcc     192
Glu Tyr Gln Gly Ile Pro His Pro Asp Asp Val Lys Ala Lys Gln Ala
    50                  55                  60

aag atg gcg ccc tcc aga atg gac agc ctt cgg ggc cag aca gac ctg     240
Lys Met Ala Pro Ser Arg Met Asp Ser Leu Arg Gly Gln Thr Asp Leu
65                  70                  75                  80

atg gct gag agg ctg gaa gat gag gag cag ttg gcc cac cag tac gag     288
Met Ala Glu Arg Leu Glu Asp Glu Glu Gln Leu Ala His Gln Tyr Glu
                85                  90                  95

acc atc atg gat gag tgt ggc cat ggc cgc ttc cag tgg atc ctc ttt     336
Thr Ile Met Asp Glu Cys Gly His Gly Arg Phe Gln Trp Ile Leu Phe
            100                 105                 110

ttc gtc ttg ggt ttg gcc ctg atg gcc gat ggg gtg gaa gtg ttc gtg     384
Phe Val Leu Gly Leu Ala Leu Met Ala Asp Gly Val Glu Val Phe Val
        115                 120                 125

gtg agt ttt gcc ctg ccc agt gca gag aag gac atg tgt ctg tcc agt     432
Val Ser Phe Ala Leu Pro Ser Ala Glu Lys Asp Met Cys Leu Ser Ser
    130                 135                 140

tcc aaa aaa gga atg cta ggg atg ata gtc tac ttg gga atg atg gcg     480
Ser Lys Lys Gly Met Leu Gly Met Ile Val Tyr Leu Gly Met Met Ala
145                 150                 155                 160

ggc gcc ttc atc ctg gga ggc ctg gct gat aag ctg gga agg aag cga     528
Gly Ala Phe Ile Leu Gly Gly Leu Ala Asp Lys Leu Gly Arg Lys Arg
                165                 170                 175

gtc ctc agc atg tct ctg gcc gtc aat gcc tcc ttc gcc tcc ctc tct     576
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Val Leu Ser Met Ser Leu Ala Val Asn Ala Ser Phe Ala Ser Leu Ser
            180                 185                 190

tcc ttc gtg cag gga tat gga gcc ttc ctc ttc tgc cga ctc atc tca      624
Ser Phe Val Gln Gly Tyr Gly Ala Phe Leu Phe Cys Arg Leu Ile Ser
        195                 200                 205

ggc atc ggt att ggg ggt gct cta ccg att gtt ttt gcc tat ttt tct      672
Gly Ile Gly Ile Gly Gly Ala Leu Pro Ile Val Phe Ala Tyr Phe Ser
    210                 215                 220

gaa ttc ttg tct cgg gag aag cga gga gaa cac ctc agt tgg ctg ggc      720
Glu Phe Leu Ser Arg Glu Lys Arg Gly Glu His Leu Ser Trp Leu Gly
225                 230                 235                 240

atc ttc tgg atg act ggg ggc ctg tac gca tct gcc atg gcc tgg agc      768
Ile Phe Trp Met Thr Gly Gly Leu Tyr Ala Ser Ala Met Ala Trp Ser
                245                 250                 255

atc atc cca cac tat ggc tgg ggc ttc agc atg ggg acc aat tac cac      816
Ile Ile Pro His Tyr Gly Trp Gly Phe Ser Met Gly Thr Asn Tyr His
            260                 265                 270

ttc cat agc tgg aga gtg ttt gtc atc gtc tgt gct ctg ccc tgc acc      864
Phe His Ser Trp Arg Val Phe Val Ile Val Cys Ala Leu Pro Cys Thr
        275                 280                 285

gtg tcc atg gtg gcc ctg aag ttc atg cca gag agc cca agg ttt ctg      912
Val Ser Met Val Ala Leu Lys Phe Met Pro Glu Ser Pro Arg Phe Leu
    290                 295                 300

cta gag atg ggc aaa cat gat gaa gcc tgg atg att ctc aag caa gtc      960
Leu Glu Met Gly Lys His Asp Glu Ala Trp Met Ile Leu Lys Gln Val
305                 310                 315                 320

cat gac acc aac atg aga gct aag ggg acc cca gag aaa gtg ttc acg     1008
His Asp Thr Asn Met Arg Ala Lys Gly Thr Pro Glu Lys Val Phe Thr
                325                 330                 335

gtt tcc aac atc aaa act ccc aag caa atg gat gaa ttc att gag atc     1056
Val Ser Asn Ile Lys Thr Pro Lys Gln Met Asp Glu Phe Ile Glu Ile
            340                 345                 350

caa agt tca aca gga acc tgg tac cag cgc tgg ctg gtc aga ttc aag     1104
Gln Ser Ser Thr Gly Thr Trp Tyr Gln Arg Trp Leu Val Arg Phe Lys
        355                 360                 365

acc att ttc aag cag gtc tgg gat aat gcc ctg tac tgt gtg atg ggg     1152
Thr Ile Phe Lys Gln Val Trp Asp Asn Ala Leu Tyr Cys Val Met Gly
    370                 375                 380

ccc tac aga atg aat aca ctg att ctg gcc gtg gtt tgg ttt gcc atg     1200
Pro Tyr Arg Met Asn Thr Leu Ile Leu Ala Val Val Trp Phe Ala Met
385                 390                 395                 400

gca ttc agt tac tat gga ctg aca gtt tgg ttt cct gat atg atc cgc     1248
Ala Phe Ser Tyr Tyr Gly Leu Thr Val Trp Phe Pro Asp Met Ile Arg
                405                 410                 415

tat ttt caa gat gaa gaa tac aag tct aaa atg aag gtg ttt ttt ggt     1296
Tyr Phe Gln Asp Glu Glu Tyr Lys Ser Lys Met Lys Val Phe Phe Gly
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                420                 425                 430

gag cat gtg tac ggc gcc aca atc aac ttc acg atg gaa aat cag atc    1344
Glu His Val Tyr Gly Ala Thr Ile Asn Phe Thr Met Glu Asn Gln Ile
        435                 440                 445

cac caa cat ggg aaa ctt gtg aat gat aag ttc aca aga atg tac ttt    1392
His Gln His Gly Lys Leu Val Asn Asp Lys Phe Thr Arg Met Tyr Phe
    450                 455                 460

aaa cat gta ctc ttt gag gac aca ttc ttt gac gag tgc tat ttt gaa    1440
Lys His Val Leu Phe Glu Asp Thr Phe Phe Asp Glu Cys Tyr Phe Glu
465                 470                 475                 480

gac gta aca tca aca gat acc tac ttc aaa aat tgt acc att gaa tca    1488
Asp Val Thr Ser Thr Asp Thr Tyr Phe Lys Asn Cys Thr Ile Glu Ser
                485                 490                 495

acc atc ttt tac aac aca gac ctc tac gag cac aag ttc atc aac tgt    1536
Thr Ile Phe Tyr Asn Thr Asp Leu Tyr Glu His Lys Phe Ile Asn Cys
            500                 505                 510

cgg ttt atc aac tcc acc ttc ctg gag cag aag gag ggc tgc cac atg    1584
Arg Phe Ile Asn Ser Thr Phe Leu Glu Gln Lys Glu Gly Cys His Met
        515                 520                 525

gac ttg gag caa gat aat gac ttc ctg att tac ctc gtc agc ttc ctg    1632
Asp Leu Glu Gln Asp Asn Asp Phe Leu Ile Tyr Leu Val Ser Phe Leu
    530                 535                 540

ggc agc ctg tct gtc tta ccc ggg aac atc att tct gcc ctg ctc atg    1680
Gly Ser Leu Ser Val Leu Pro Gly Asn Ile Ile Ser Ala Leu Leu Met
545                 550                 555                 560

gat aga att gga agg ctc aag atg att ggt ggc tcc atg cta atc tct    1728
Asp Arg Ile Gly Arg Leu Lys Met Ile Gly Gly Ser Met Leu Ile Ser
                565                 570                 575

gca gtc tgc tgc ttc ttc ctg ttt ttt ggc aac agt gag tct gca atg    1776
Ala Val Cys Cys Phe Phe Leu Phe Phe Gly Asn Ser Glu Ser Ala Met
            580                 585                 590

atc ggc tgg cag tgc ctg ttc tgt ggg aca agc att gca gcc tgg aat    1824
Ile Gly Trp Gln Cys Leu Phe Cys Gly Thr Ser Ile Ala Ala Trp Asn
        595                 600                 605

gct ctg gat gtg atc aca gtg gag ctg tat ccc acc aac cag aga gca    1872
Ala Leu Asp Val Ile Thr Val Glu Leu Tyr Pro Thr Asn Gln Arg Ala
    610                 615                 620

aca gcc ttc ggc att ctc aat gga tta tgc aaa ttt ggc gcc atc ctg    1920
Thr Ala Phe Gly Ile Leu Asn Gly Leu Cys Lys Phe Gly Ala Ile Leu
625                 630                 635                 640

gga aac acc atc ttt gct tct ttt gtt ggg ata acc aaa gtg gtc ccc    1968
Gly Asn Thr Ile Phe Ala Ser Phe Val Gly Ile Thr Lys Val Val Pro
                645                 650                 655

atc ctt ctg gct gct gct tct ctg gtt ggg ggt ggc ctg att gcc ctt    2016
Ile Leu Leu Ala Ala Ala Ser Leu Val Gly Gly Gly Leu Ile Ala Leu
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675 680

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Ser Asp Val Thr Glu Gly His Asp Glu Glu Asp Glu Ile Tyr Glu Gly
35 40 45

Glu Tyr Gln Gly Ile Pro His Pro Asp Asp Val Lys Ala Lys Gln Ala
50 55 60

Lys Met Ala Pro Ser Arg Met Asp Ser Leu Arg Gly Gln Thr Asp Leu
65 70 75 80

Met Ala Glu Arg Leu Glu Asp Glu Glu Gln Leu Ala His Gln Tyr Glu
85 90 95

Thr Ile Met Asp Glu Cys Gly His Gly Arg Phe Gln Trp Ile Leu Phe
100 105 110

Phe Val Leu Gly Leu Ala Leu Met Ala Asp Gly Val Glu Val Phe Val
115 120 125

Val Ser Phe Ala Leu Pro Ser Ala Glu Lys Asp Met Cys Leu Ser Ser
130 135 140

Ser Lys Lys Gly Met Leu Gly Met Ile Val Tyr Leu Gly Met Met Ala
145 150 155 160

Gly Ala Phe Ile Leu Gly Gly Leu Ala Asp Lys Leu Gly Arg Lys Arg
165 170 175

Val Leu Ser Met Ser Leu Ala Val Asn Ala Ser Phe Ala Ser Leu Ser
180 185 190

Ser Phe Val Gln Gly Tyr Gly Ala Phe Leu Phe Cys Arg Leu Ile Ser
195 200 205

Gly Ile Gly Ile Gly Gly Ala Leu Pro Ile Val Phe Ala Tyr Phe Ser
210 215 220

Glu Phe Leu Ser Arg Glu Lys Arg Gly Glu His Leu Ser Trp Leu Gly
225 230 235 240

Ile Phe Trp Met Thr Gly Gly Leu Tyr Ala Ser Ala Met Ala Trp Ser
245 250 255

Ile Ile Pro His Tyr Gly Trp Gly Phe Ser Met Gly Thr Asn Tyr His

260 265 270

Phe His Ser Trp Arg Val Phe Val Ile Val Cys Ala Leu Pro Cys Thr
275 280 285

Val Ser Met Val Ala Leu Lys Phe Met Pro Glu Ser Pro Arg Phe Leu
290 295 300

Leu Glu Met Gly Lys His Asp Glu Ala Trp Met Ile Leu Lys Gln Val
305 310 315 320

His Asp Thr Asn Met Arg Ala Lys Gly Thr Pro Glu Lys Val Phe Thr
325 330 335

Val Ser Asn Ile Lys Thr Pro Lys Gln Met Asp Glu Phe Ile Glu Ile
340 345 350

Gln Ser Ser Thr Gly Thr Trp Tyr Gln Arg Trp Leu Val Arg Phe Lys
355 360 365

Thr Ile Phe Lys Gln Val Trp Asp Asn Ala Leu Tyr Cys Val Met Gly
370 375 380

Pro Tyr Arg Met Asn Thr Leu Ile Leu Ala Val Val Trp Phe Ala Met
385 390 395 400

Ala Phe Ser Tyr Tyr Gly Leu Thr Val Trp Phe Pro Asp Met Ile Arg
405 410 415

Tyr Phe Gln Asp Glu Glu Tyr Lys Ser Lys Met Lys Val Phe Phe Gly
420 425 430

Glu His Val Tyr Gly Ala Thr Ile Asn Phe Thr Met Glu Asn Gln Ile
435 440 445

His Gln His Gly Lys Leu Val Asn Asp Lys Phe Thr Arg Met Tyr Phe
450 455 460

Lys His Val Leu Phe Glu Asp Thr Phe Phe Asp Glu Cys Tyr Phe Glu
465 470 475 480

Asp Val Thr Ser Thr Asp Thr Tyr Phe Lys Asn Cys Thr Ile Glu Ser
485 490 495

Thr Ile Phe Tyr Asn Thr Asp Leu Tyr Glu His Lys Phe Ile Asn Cys
500 505 510

Arg Phe Ile Asn Ser Thr Phe Leu Glu Gln Lys Glu Gly Cys His Met
515 520 525

Asp Leu Glu Gln Asp Asn Asp Phe Leu Ile Tyr Leu Val Ser Phe Leu
530 535 540

Gly Ser Leu Ser Val Leu Pro Gly Asn Ile Ile Ser Ala Leu Leu Met
545 550 555 560

Asp Arg Ile Gly Arg Leu Lys Met Ile Gly Gly Ser Met Leu Ile Ser
565 570 575

Ala Val Cys Cys Phe Phe Leu Phe Phe Gly Asn Ser Glu Ser Ala Met
580 585 590

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Ile Gly Trp Gln Cys Leu Phe Cys Gly Thr Ser Ile Ala Ala Trp Asn
        595                 600                 605

Ala Leu Asp Val Ile Thr Val Glu Leu Tyr Pro Thr Asn Gln Arg Ala
    610                 615                 620

Thr Ala Phe Gly Ile Leu Asn Gly Leu Cys Lys Phe Gly Ala Ile Leu
625                 630                 635                 640

Gly Asn Thr Ile Phe Ala Ser Phe Val Gly Ile Thr Lys Val Val Pro
                645                 650                 655

Ile Leu Leu Ala Ala Ala Ser Leu Val Gly Gly Gly Leu Ile Ala Leu
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Arg Leu Pro Glu Thr Arg Glu Gln Val Leu Met
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1               5                   10                  15

gac att gcc aga gag gtg aag aaa caa aca gta aag aag gtg aat caa      96
Asp Ile Ala Arg Glu Val Lys Lys Gln Thr Val Lys Lys Val Asn Gln
            20                  25                  30

gct gtg gac cga gcc cag gat gaa tac acc cag agg tcc tac agt cgg     144
Ala Val Asp Arg Ala Gln Asp Glu Tyr Thr Gln Arg Ser Tyr Ser Arg
        35                  40                  45

ttc caa gat gaa gaa gat gat gat gac tac tac ccg gct gga gaa acc     192
Phe Gln Asp Glu Glu Asp Asp Asp Asp Tyr Tyr Pro Ala Gly Glu Thr
    50                  55                  60

tat aat ggt gag gcc aac gat gac gaa ggc tca agt gaa gcc act gag     240
Tyr Asn Gly Glu Ala Asn Asp Asp Glu Gly Ser Ser Glu Ala Thr Glu
65                  70                  75                  80

ggg cat gat gaa gat gat gag atc tat gag ggg gag tat cag ggc atc     288
Gly His Asp Glu Asp Asp Glu Ile Tyr Glu Gly Glu Tyr Gln Gly Ile
                85                  90                  95

ccc agt atg aac caa gcg aag gac agc atc gtg tca gtg ggg cag ccc     336
Pro Ser Met Asn Gln Ala Lys Asp Ser Ile Val Ser Val Gly Gln Pro
            100                 105                 110

aag ggc gat gag tac aag gac cga cgg gag ctg gaa tca gaa agg aga     384
Lys Gly Asp Glu Tyr Lys Asp Arg Arg Glu Leu Glu Ser Glu Arg Arg
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        115                 120                 125

gct gac gag gaa gag tta gcc cag cag tat gag ctg ata atc caa gaa      432
Ala Asp Glu Glu Glu Leu Ala Gln Gln Tyr Glu Leu Ile Ile Gln Glu
    130                 135                 140

tgc ggt cat ggt cgt ttt cag tgg gcc ctt ttc ttc gtc ctg ggc atg      480
Cys Gly His Gly Arg Phe Gln Trp Ala Leu Phe Phe Val Leu Gly Met
145                 150                 155                 160

gct ctt atg gca gac ggt gta gag gtg ttt gtc gtt ggc ttc gtg tta      528
Ala Leu Met Ala Asp Gly Val Glu Val Phe Val Val Gly Phe Val Leu
                165                 170                 175

ccc agt gct gag aca gac ctc tgc atc cca aat tca gga tct gga tgg      576
Pro Ser Ala Glu Thr Asp Leu Cys Ile Pro Asn Ser Gly Ser Gly Trp
            180                 185                 190

cta ggc agc ata gtg tac ctc ggg atg atg gtg ggg gcg ttc ttc tgg      624
Leu Gly Ser Ile Val Tyr Leu Gly Met Met Val Gly Ala Phe Phe Trp
        195                 200                 205

gga gga ctg gca gac aaa gtg gga agg aaa cag tct ctt ctg att tgc      672
Gly Gly Leu Ala Asp Lys Val Gly Arg Lys Gln Ser Leu Leu Ile Cys
    210                 215                 220

atg tct gtc aac gga ttc ttt gcc ttc ctt tct tca ttt gtc caa ggt      720
Met Ser Val Asn Gly Phe Phe Ala Phe Leu Ser Ser Phe Val Gln Gly
225                 230                 235                 240

tat ggc ttc ttt ctc ttc tgt cgc tta ctt tct gga ttc ggg att gga      768
Tyr Gly Phe Phe Leu Phe Cys Arg Leu Leu Ser Gly Phe Gly Ile Gly
                245                 250                 255

gga gcc ata ccc act gtg ttc tcg tac ttt gct gaa gtc ctg gcc cgg      816
Gly Ala Ile Pro Thr Val Phe Ser Tyr Phe Ala Glu Val Leu Ala Arg
            260                 265                 270

gaa aag cgg ggc gaa cac ttg agc tgg ctc tgc atg ttc tgg atg atc      864
Glu Lys Arg Gly Glu His Leu Ser Trp Leu Cys Met Phe Trp Met Ile
        275                 280                 285

ggt ggc atc tac gcc tct gcc atg gcc tgg gcc atc atc ccg cac tac      912
Gly Gly Ile Tyr Ala Ser Ala Met Ala Trp Ala Ile Ile Pro His Tyr
    290                 295                 300

ggg tgg agc ttc agc atg gga tcg gcc tac cag ttt cac agt tgg cgt      960
Gly Trp Ser Phe Ser Met Gly Ser Ala Tyr Gln Phe His Ser Trp Arg
305                 310                 315                 320

gtg ttt gtc atc gtc tgt gca ctc ccc tgt gtc tcc tcc gtg gtg gcc     1008
Val Phe Val Ile Val Cys Ala Leu Pro Cys Val Ser Ser Val Val Ala
                325                 330                 335

ctc aca ttc atg cct gaa agc cca cga ttc ttg ttg gag gtt gga aaa     1056
Leu Thr Phe Met Pro Glu Ser Pro Arg Phe Leu Leu Glu Val Gly Lys
            340                 345                 350

cat gat gaa gct tgg atg att ctg aag tta att cat gac acc aac atg     1104
His Asp Glu Ala Trp Met Ile Leu Lys Leu Ile His Asp Thr Asn Met
        355                 360                 365
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aga gcc cgg ggt cag cct gag aag gtc ttc acg gta aac aaa ata aaa     1152
Arg Ala Arg Gly Gln Pro Glu Lys Val Phe Thr Val Asn Lys Ile Lys
    370                 375                 380

act cct aaa caa ata gat gag ctg att gaa att gag agt gac aca gga     1200
Thr Pro Lys Gln Ile Asp Glu Leu Ile Glu Ile Glu Ser Asp Thr Gly
385                 390                 395                 400

aca tgg tat agg agg tgt ttt gtt cgg atc cgc acc gag ctg tac gga     1248
Thr Trp Tyr Arg Arg Cys Phe Val Arg Ile Arg Thr Glu Leu Tyr Gly
                405                 410                 415

att tgg ttg act ttt atg aga tgt ttc aac tac cca gtc agg gat aat     1296
Ile Trp Leu Thr Phe Met Arg Cys Phe Asn Tyr Pro Val Arg Asp Asn
            420                 425                 430

aca ata aag ctt aca att gtt tgg ttc acc ctg tcc ttt ggg tac tat     1344
Thr Ile Lys Leu Thr Ile Val Trp Phe Thr Leu Ser Phe Gly Tyr Tyr
        435                 440                 445

gga tta tcc gtt tgg ttc cct gat gtc att aaa cct ctg cag tcc gat     1392
Gly Leu Ser Val Trp Phe Pro Asp Val Ile Lys Pro Leu Gln Ser Asp
    450                 455                 460

gaa tat gca ttg cta acc aga aat gtg gag aga gat aaa tat gca aat     1440
Glu Tyr Ala Leu Leu Thr Arg Asn Val Glu Arg Asp Lys Tyr Ala Asn
465                 470                 475                 480

ttc act att aac ttt aca atg gaa aat cag att cat act gga atg gaa     1488
Phe Thr Ile Asn Phe Thr Met Glu Asn Gln Ile His Thr Gly Met Glu
                485                 490                 495

tac gac aat ggc aga ttc ata ggg gtc aag ttc aaa tct gta act ttc     1536
Tyr Asp Asn Gly Arg Phe Ile Gly Val Lys Phe Lys Ser Val Thr Phe
            500                 505                 510

aaa gac tct gtt ttt aag tcc tgc acc ttt gag gat gta act tca gtg     1584
Lys Asp Ser Val Phe Lys Ser Cys Thr Phe Glu Asp Val Thr Ser Val
        515                 520                 525

aac acc tac ttc aag aac tgc aca ttt att gac act gtt ttt gac aac     1632
Asn Thr Tyr Phe Lys Asn Cys Thr Phe Ile Asp Thr Val Phe Asp Asn
    530                 535                 540

aca gat ttt gag cca tat aaa ttc att gac agt gaa ttt aaa aac tgc     1680
Thr Asp Phe Glu Pro Tyr Lys Phe Ile Asp Ser Glu Phe Lys Asn Cys
545                 550                 555                 560

tcg ttt ttt cac aac aag acg gga tgt cag att acc ttt gat gat gac     1728
Ser Phe Phe His Asn Lys Thr Gly Cys Gln Ile Thr Phe Asp Asp Asp
                565                 570                 575

tat agt gcc tac tgg att tat ttt gtc aac ttt ctg ggg aca ttg gca     1776
Tyr Ser Ala Tyr Trp Ile Tyr Phe Val Asn Phe Leu Gly Thr Leu Ala
            580                 585                 590

gta ttg cca ggg aac att gtg tct gct ctg ctg atg gac aga att ggg     1824
Val Leu Pro Gly Asn Ile Val Ser Ala Leu Leu Met Asp Arg Ile Gly
        595                 600                 605
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cgc tta aca atg cta ggt ggc tct atg gtg ctt tcg ggg atc agc tgt     1872
Arg Leu Thr Met Leu Gly Gly Ser Met Val Leu Ser Gly Ile Ser Cys
    610                 615                 620

ttc ttc ctt tgg ttc ggc acc agt gaa tcc atg atg ata ggc atg ctg     1920
Phe Phe Leu Trp Phe Gly Thr Ser Glu Ser Met Met Ile Gly Met Leu
625                 630                 635                 640

tgt ctg tac aat gga ttg acc atc tca gcc tgg aac tct ctt gac gtg     1968
Cys Leu Tyr Asn Gly Leu Thr Ile Ser Ala Trp Asn Ser Leu Asp Val
                645                 650                 655

gtc act gtg gaa ctg tac ccc aca gac cgg agg gca aca ggc ttt ggc     2016
Val Thr Val Glu Leu Tyr Pro Thr Asp Arg Arg Ala Thr Gly Phe Gly
            660                 665                 670

ttc tta aat gcg cta tgc aag gca gca gcc gtc ctg gga aac tta ata     2064
Phe Leu Asn Ala Leu Cys Lys Ala Ala Ala Val Leu Gly Asn Leu Ile
        675                 680                 685

ttt ggc tct ctg gtc agc atc acc aaa tca atc ccc atc ctg ctg gct     2112
Phe Gly Ser Leu Val Ser Ile Thr Lys Ser Ile Pro Ile Leu Leu Ala
    690                 695                 700

tct act gtg ctc gtg tgt gga gga ctc gtt ggg ctg tgc ctg cct gac     2160
Ser Thr Val Leu Val Cys Gly Gly Leu Val Gly Leu Cys Leu Pro Asp
705                 710                 715                 720

aca cga acc cag gtt ctg atg taa                                     2184
Thr Arg Thr Gln Val Leu Met
                725
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Met Glu Asp Ser Tyr Lys Asp Arg Thr Ser Leu Met Lys Gly Ala Lys
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Asp Ile Ala Arg Glu Val Lys Lys Gln Thr Val Lys Lys Val Asn Gln
            20                  25                  30

Ala Val Asp Arg Ala Gln Asp Glu Tyr Thr Gln Arg Ser Tyr Ser Arg
        35                  40                  45

Phe Gln Asp Glu Glu Asp Asp Asp Asp Tyr Tyr Pro Ala Gly Glu Thr
    50                  55                  60

Tyr Asn Gly Glu Ala Asn Asp Asp Glu Gly Ser Ser Glu Ala Thr Glu
65                  70                  75                  80

Gly His Asp Glu Asp Asp Glu Ile Tyr Glu Gly Glu Tyr Gln Gly Ile
                85                  90                  95

Pro Ser Met Asn Gln Ala Lys Asp Ser Ile Val Ser Val Gly Gln Pro
            100                 105                 110

Lys Gly Asp Glu Tyr Lys Asp Arg Arg Glu Leu Glu Ser Glu Arg Arg
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115 120 125

Ala Asp Glu Glu Glu Leu Ala Gln Gln Tyr Glu Leu Ile Ile Gln Glu
130 135 140

Cys Gly His Gly Arg Phe Gln Trp Ala Leu Phe Phe Val Leu Gly Met
145 150 155 160

Ala Leu Met Ala Asp Gly Val Glu Val Phe Val Val Gly Phe Val Leu
165 170 175

Pro Ser Ala Glu Thr Asp Leu Cys Ile Pro Asn Ser Gly Ser Gly Trp
180 185 190

Leu Gly Ser Ile Val Tyr Leu Gly Met Met Val Gly Ala Phe Phe Trp
195 200 205

Gly Gly Leu Ala Asp Lys Val Gly Arg Lys Gln Ser Leu Leu Ile Cys
210 215 220

Met Ser Val Asn Gly Phe Phe Ala Phe Leu Ser Ser Phe Val Gln Gly
225 230 235 240

Tyr Gly Phe Phe Leu Phe Cys Arg Leu Leu Ser Gly Phe Gly Ile Gly
245 250 255

Gly Ala Ile Pro Thr Val Phe Ser Tyr Phe Ala Glu Val Leu Ala Arg
260 265 270

Glu Lys Arg Gly Glu His Leu Ser Trp Leu Cys Met Phe Trp Met Ile
275 280 285

Gly Gly Ile Tyr Ala Ser Ala Met Ala Trp Ala Ile Ile Pro His Tyr
290 295 300

Gly Trp Ser Phe Ser Met Gly Ser Ala Tyr Gln Phe His Ser Trp Arg
305 310 315 320

Val Phe Val Ile Val Cys Ala Leu Pro Cys Val Ser Ser Val Val Ala
325 330 335

Leu Thr Phe Met Pro Glu Ser Pro Arg Phe Leu Leu Glu Val Gly Lys
340 345 350

His Asp Glu Ala Trp Met Ile Leu Lys Leu Ile His Asp Thr Asn Met
355 360 365

Arg Ala Arg Gly Gln Pro Glu Lys Val Phe Thr Val Asn Lys Ile Lys
370 375 380

Thr Pro Lys Gln Ile Asp Glu Leu Ile Glu Ile Glu Ser Asp Thr Gly
385 390 395 400

Thr Trp Tyr Arg Arg Cys Phe Val Arg Ile Arg Thr Glu Leu Tyr Gly
405 410 415

Ile Trp Leu Thr Phe Met Arg Cys Phe Asn Tyr Pro Val Arg Asp Asn
420 425 430

Thr Ile Lys Leu Thr Ile Val Trp Phe Thr Leu Ser Phe Gly Tyr Tyr
435 440 445

Gly Leu Ser Val Trp Phe Pro Asp Val Ile Lys Pro Leu Gln Ser Asp
450 455 460

Glu Tyr Ala Leu Leu Thr Arg Asn Val Glu Arg Asp Lys Tyr Ala Asn
465 470 475 480

Phe Thr Ile Asn Phe Thr Met Glu Asn Gln Ile His Thr Gly Met Glu
485 490 495

Tyr Asp Asn Gly Arg Phe Ile Gly Val Lys Phe Lys Ser Val Thr Phe
500 505 510

Lys Asp Ser Val Phe Lys Ser Cys Thr Phe Glu Asp Val Thr Ser Val
515 520 525

Asn Thr Tyr Phe Lys Asn Cys Thr Phe Ile Asp Thr Val Phe Asp Asn
530 535 540

Thr Asp Phe Glu Pro Tyr Lys Phe Ile Asp Ser Glu Phe Lys Asn Cys
545 550 555 560

Ser Phe Phe His Asn Lys Thr Gly Cys Gln Ile Thr Phe Asp Asp Asp
565 570 575

Tyr Ser Ala Tyr Trp Ile Tyr Phe Val Asn Phe Leu Gly Thr Leu Ala
580 585 590

Val Leu Pro Gly Asn Ile Val Ser Ala Leu Leu Met Asp Arg Ile Gly
595 600 605

Arg Leu Thr Met Leu Gly Gly Ser Met Val Leu Ser Gly Ile Ser Cys
610 615 620

Phe Phe Leu Trp Phe Gly Thr Ser Glu Ser Met Met Ile Gly Met Leu
625 630 635 640

Cys Leu Tyr Asn Gly Leu Thr Ile Ser Ala Trp Asn Ser Leu Asp Val
645 650 655

Val Thr Val Glu Leu Tyr Pro Thr Asp Arg Arg Ala Thr Gly Phe Gly
660 665 670

Phe Leu Asn Ala Leu Cys Lys Ala Ala Ala Val Leu Gly Asn Leu Ile
675 680 685

Phe Gly Ser Leu Val Ser Ile Thr Lys Ser Ile Pro Ile Leu Leu Ala
690 695 700

Ser Thr Val Leu Val Cys Gly Gly Leu Val Gly Leu Cys Leu Pro Asp
705 710 715 720

Thr Arg Thr Gln Val Leu Met
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Met Glu Glu Asp Leu Phe Gln Leu Arg Gln Leu Pro Val Val Lys Phe
1               5                   10                  15

cgt cgc aca ggc gag agt gca agg tca gag gac gac acg gct tca gga       96
Arg Arg Thr Gly Glu Ser Ala Arg Ser Glu Asp Asp Thr Ala Ser Gly
            20                  25                  30

gag cat gaa gtc cag att gaa ggg gtc cac gtg ggc cta gag gct gtg      144
Glu His Glu Val Gln Ile Glu Gly Val His Val Gly Leu Glu Ala Val
        35                  40                  45

gag ctg gat gat ggg gca gct gtg ccc aag gag ttt gcc aat ccc act      192
Glu Leu Asp Asp Gly Ala Ala Val Pro Lys Glu Phe Ala Asn Pro Thr
    50                  55                  60

gat gat act ttc atg gtg gaa gat gca gtg gaa gcc att ggc ttt gga      240
Asp Asp Thr Phe Met Val Glu Asp Ala Val Glu Ala Ile Gly Phe Gly
65                  70                  75                  80

aaa ttt cag tgg aag ctg tct gtt ctc act ggc ttg gct tgg atg gct      288
Lys Phe Gln Trp Lys Leu Ser Val Leu Thr Gly Leu Ala Trp Met Ala
                85                  90                  95

gat gcc atg gag atg atg atc ctc agc atc ctg gca cca cag ctg cat      336
Asp Ala Met Glu Met Met Ile Leu Ser Ile Leu Ala Pro Gln Leu His
            100                 105                 110

tgc gag tgg agg ctc cca agc tgg cag gtg gca ttg ctg acc tcg gtg      384
Cys Glu Trp Arg Leu Pro Ser Trp Gln Val Ala Leu Leu Thr Ser Val
        115                 120                 125

gtc ttt gta ggc atg atg tcc agc tcc acg ctc tgg gga aat atc tca      432
Val Phe Val Gly Met Met Ser Ser Ser Thr Leu Trp Gly Asn Ile Ser
    130                 135                 140

gac cag tac ggc agg aaa aca ggg ctg aag atc agc gtg ctg tgg act      480
Asp Gln Tyr Gly Arg Lys Thr Gly Leu Lys Ile Ser Val Leu Trp Thr
145                 150                 155                 160

ctg tac tat ggc atc ctt agt gca ttt gcg ccc gtg tat agc tgg atc      528
Leu Tyr Tyr Gly Ile Leu Ser Ala Phe Ala Pro Val Tyr Ser Trp Ile
                165                 170                 175

ctg gtg ctc cgg ggc ctg gtg ggc ttc ggg atc gga gga gtt ccc cag      576
Leu Val Leu Arg Gly Leu Val Gly Phe Gly Ile Gly Gly Val Pro Gln
            180                 185                 190

tcg gtg acg ctg tat gcc gag ttc ctt ccc atg aaa gcc aga gct aaa      624
Ser Val Thr Leu Tyr Ala Glu Phe Leu Pro Met Lys Ala Arg Ala Lys
        195                 200                 205

tgt att ttg ctg att gag gta ttc tgg gcc atc ggg aca gtg ttc gag      672
Cys Ile Leu Leu Ile Glu Val Phe Trp Ala Ile Gly Thr Val Phe Glu
    210                 215                 220
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gtc gtc ctg gct gtg ttc gtg atg ccc agc ctg ggc tgg cgt tgg ctg      720
Val Val Leu Ala Val Phe Val Met Pro Ser Leu Gly Trp Arg Trp Leu
225                 230                 235                 240

ctc atc ctc tca gct gtc ccg ctc ctc ctc ttt gcc gtg ctg tgt ttc      768
Leu Ile Leu Ser Ala Val Pro Leu Leu Leu Phe Ala Val Leu Cys Phe
                245                 250                 255

tgg ctg cct gaa agt gca agg tat gat gtg ctg tca ggg aac cag gaa      816
Trp Leu Pro Glu Ser Ala Arg Tyr Asp Val Leu Ser Gly Asn Gln Glu
            260                 265                 270

aag gca atc gcc acc tta aag agg ata gca act gaa aac gga gct ccc      864
Lys Ala Ile Ala Thr Leu Lys Arg Ile Ala Thr Glu Asn Gly Ala Pro
        275                 280                 285

atg ccg ctg ggg aaa ctc atc atc tcc aga cag gaa gac cga ggc aaa      912
Met Pro Leu Gly Lys Leu Ile Ile Ser Arg Gln Glu Asp Arg Gly Lys
    290                 295                 300

atg agg gac ctt ttc aca ccc cat ttt aga tgg aca act ttg ctg ctg      960
Met Arg Asp Leu Phe Thr Pro His Phe Arg Trp Thr Thr Leu Leu Leu
305                 310                 315                 320

tgg ttt ata tgg ttt tcc aat gca ttc tct tac tac ggg tta gtt cta     1008
Trp Phe Ile Trp Phe Ser Asn Ala Phe Ser Tyr Tyr Gly Leu Val Leu
                325                 330                 335

ctc acc aca gaa ctc ttc cag gca gga gat gtc tgc ggc atc tcc agt     1056
Leu Thr Thr Glu Leu Phe Gln Ala Gly Asp Val Cys Gly Ile Ser Ser
            340                 345                 350

cgg aag aag gct gta gag gca aaa tgc agc ctg gcc tgc gag tac ctg     1104
Arg Lys Lys Ala Val Glu Ala Lys Cys Ser Leu Ala Cys Glu Tyr Leu
        355                 360                 365

agt gag gag gat tac atg gac ttg ctg tgg acc acc ctc tct gag ttt     1152
Ser Glu Glu Asp Tyr Met Asp Leu Leu Trp Thr Thr Leu Ser Glu Phe
    370                 375                 380

cca ggt gtc ctt gtg act ctg tgg att att gac cgc ctg ggg cgc aag     1200
Pro Gly Val Leu Val Thr Leu Trp Ile Ile Asp Arg Leu Gly Arg Lys
385                 390                 395                 400

aag acc atg gcc ctg tgc ttt gtc atc ttc tcc ttc tgc agc ctc ctg     1248
Lys Thr Met Ala Leu Cys Phe Val Ile Phe Ser Phe Cys Ser Leu Leu
                405                 410                 415

ctg ttt atc tgt gtt gga aga aat gtg ctc act ctg tta ctc ttc att     1296
Leu Phe Ile Cys Val Gly Arg Asn Val Leu Thr Leu Leu Leu Phe Ile
            420                 425                 430

gca aga gcg ttt att tct gga ggc ttt caa gcg gca tat gtt tac aca     1344
Ala Arg Ala Phe Ile Ser Gly Gly Phe Gln Ala Ala Tyr Val Tyr Thr
        435                 440                 445

cct gag gtc tac ccc acg gca acg cgg gcc ctc ggc ctg ggc acc tgc     1392
Pro Glu Val Tyr Pro Thr Ala Thr Arg Ala Leu Gly Leu Gly Thr Cys
    450                 455                 460
```

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agc ggc atg gca aga gtg ggt gct ctc atc act ccg ttc atc gcc cag     1440
Ser Gly Met Ala Arg Val Gly Ala Leu Ile Thr Pro Phe Ile Ala Gln
465                 470                 475                 480

gtg atg ctg gaa tcc tct gtg tac ctg act ctg gca gtt tac agt ggc     1488
Val Met Leu Glu Ser Ser Val Tyr Leu Thr Leu Ala Val Tyr Ser Gly
                485                 490                 495

tgc tgc ctc ctg gct gcc ctg gcc tcc tgc ttt ttg ccc att gag acc     1536
Cys Cys Leu Leu Ala Ala Leu Ala Ser Cys Phe Leu Pro Ile Glu Thr
            500                 505                 510

aaa ggc cga gga ctg cag gag tcc agc cac cgg gag tgg ggc cag gag     1584
Lys Gly Arg Gly Leu Gln Glu Ser Ser His Arg Glu Trp Gly Gln Glu
        515                 520                 525

atg gtc ggc cga gga atg cac ggt gca ggt gtt acc agg tcg aac tct     1632
Met Val Gly Arg Gly Met His Gly Ala Gly Val Thr Arg Ser Asn Ser
    530                 535                 540

ggc tct cag gaa tag                                             1647
Gly Ser Gln Glu
545
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<210> 8
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<400> 8

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Met Glu Glu Asp Leu Phe Gln Leu Arg Gln Leu Pro Val Val Lys Phe
1               5                   10                  15

Arg Arg Thr Gly Glu Ser Ala Arg Ser Glu Asp Asp Thr Ala Ser Gly
            20                  25                  30

Glu His Glu Val Gln Ile Glu Gly Val His Val Gly Leu Glu Ala Val
        35                  40                  45

Glu Leu Asp Asp Gly Ala Ala Val Pro Lys Glu Phe Ala Asn Pro Thr
    50                  55                  60

Asp Asp Thr Phe Met Val Glu Asp Ala Val Glu Ala Ile Gly Phe Gly
65                  70                  75                  80

Lys Phe Gln Trp Lys Leu Ser Val Leu Thr Gly Leu Ala Trp Met Ala
                85                  90                  95

Asp Ala Met Glu Met Met Ile Leu Ser Ile Leu Ala Pro Gln Leu His
            100                 105                 110

Cys Glu Trp Arg Leu Pro Ser Trp Gln Val Ala Leu Leu Thr Ser Val
        115                 120                 125

Val Phe Val Gly Met Met Ser Ser Ser Thr Leu Trp Gly Asn Ile Ser
    130                 135                 140

Asp Gln Tyr Gly Arg Lys Thr Gly Leu Lys Ile Ser Val Leu Trp Thr
145                 150                 155                 160
```

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Leu Tyr Tyr Gly Ile Leu Ser Ala Phe Ala Pro Val Tyr Ser Trp Ile
                165                 170                 175

Leu Val Leu Arg Gly Leu Val Gly Phe Gly Ile Gly Gly Val Pro Gln
            180                 185                 190

Ser Val Thr Leu Tyr Ala Glu Phe Leu Pro Met Lys Ala Arg Ala Lys
        195                 200                 205

Cys Ile Leu Leu Ile Glu Val Phe Trp Ala Ile Gly Thr Val Phe Glu
    210                 215                 220

Val Val Leu Ala Val Phe Val Met Pro Ser Leu Gly Trp Arg Trp Leu
225                 230                 235                 240

Leu Ile Leu Ser Ala Val Pro Leu Leu Leu Phe Ala Val Leu Cys Phe
                245                 250                 255

Trp Leu Pro Glu Ser Ala Arg Tyr Asp Val Leu Ser Gly Asn Gln Glu
            260                 265                 270

Lys Ala Ile Ala Thr Leu Lys Arg Ile Ala Thr Glu Asn Gly Ala Pro
        275                 280                 285

Met Pro Leu Gly Lys Leu Ile Ile Ser Arg Gln Glu Asp Arg Gly Lys
    290                 295                 300

Met Arg Asp Leu Phe Thr Pro His Phe Arg Trp Thr Thr Leu Leu Leu
305                 310                 315                 320

Trp Phe Ile Trp Phe Ser Asn Ala Phe Ser Tyr Tyr Gly Leu Val Leu
                325                 330                 335

Leu Thr Thr Glu Leu Phe Gln Ala Gly Asp Val Cys Gly Ile Ser Ser
            340                 345                 350

Arg Lys Lys Ala Val Glu Ala Lys Cys Ser Leu Ala Cys Glu Tyr Leu
        355                 360                 365

Ser Glu Glu Asp Tyr Met Asp Leu Leu Trp Thr Thr Leu Ser Glu Phe
    370                 375                 380

Pro Gly Val Leu Val Thr Leu Trp Ile Ile Asp Arg Leu Gly Arg Lys
385                 390                 395                 400

Lys Thr Met Ala Leu Cys Phe Val Ile Phe Ser Phe Cys Ser Leu Leu
                405                 410                 415

Leu Phe Ile Cys Val Gly Arg Asn Val Leu Thr Leu Leu Leu Phe Ile
            420                 425                 430

Ala Arg Ala Phe Ile Ser Gly Gly Phe Gln Ala Ala Tyr Val Tyr Thr
        435                 440                 445

Pro Glu Val Tyr Pro Thr Ala Thr Arg Ala Leu Gly Leu Gly Thr Cys
    450                 455                 460

Ser Gly Met Ala Arg Val Gly Ala Leu Ile Thr Pro Phe Ile Ala Gln
465                 470                 475                 480

Val Met Leu Glu Ser Ser Val Tyr Leu Thr Leu Ala Val Tyr Ser Gly
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485 490 495

Cys Cys Leu Leu Ala Ala Leu Ala Ser Cys Phe Leu Pro Ile Glu Thr
500 505 510

Lys Gly Arg Gly Leu Gln Glu Ser Ser His Arg Glu Trp Gly Gln Glu
515 520 525

Met Val Gly Arg Gly Met His Gly Ala Gly Val Thr Arg Ser Asn Ser
530 535 540

Gly Ser Gln Glu
545

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tcctcagttt atcctttcca acctggctcc cccttcccag ttcccctccc tactccctgt 180

ctccctcccc aactcaccct actgaactgg gtgcagagca aagccctttt cgccctttc 240

cccatctgga cttctctggc cagttcctct tagtccgatc ccaaagacac tggaacacat 300

ttctaaaggg tcttcttgat ccctccaatt cattgagcaa agggctgaaa aagaagcaga 360

gagtaaggta gagccagtga ctcgccccca agccccatc atg gaa gaa ggc ttt 414
Met Glu Glu Gly Phe
1 5

cga gac cga gca gcg ttc atc cgt ggg gcc aaa gac att gcc aag gaa 462
Arg Asp Arg Ala Ala Phe Ile Arg Gly Ala Lys Asp Ile Ala Lys Glu
10 15 20

gtt aag aag cac gcg gcc aag aag gtg gtg aag ggt ctc gac aga gtc 510
Val Lys Lys His Ala Ala Lys Lys Val Val Lys Gly Leu Asp Arg Val
25 30 35

cag gat gaa tat tcc cga agg tcc tac tcc cgc ttt gag gag gag gag 558
Gln Asp Glu Tyr Ser Arg Arg Ser Tyr Ser Arg Phe Glu Glu Glu Glu
40 45 50

gat gat gat gac ttc cct gcc cct gct gac ggc tat tac cgc gga gaa 606
Asp Asp Asp Asp Phe Pro Ala Pro Ala Asp Gly Tyr Tyr Arg Gly Glu
55 60 65

ggg gcc cag gat gag gag gaa ggt ggc gct tcc agt gat gcc act gag 654
Gly Ala Gln Asp Glu Glu Glu Gly Gly Ala Ser Ser Asp Ala Thr Glu
70 75 80 85

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ggc cac gat gag gat gat gag atc tac gag gga gaa tat cag ggc atc      702
Gly His Asp Glu Asp Asp Glu Ile Tyr Glu Gly Glu Tyr Gln Gly Ile
                90                  95                  100

ccc cgg gca gag tct ggg ggc aaa ggc gaa cgg atg gca gat ggg gca      750
Pro Arg Ala Glu Ser Gly Gly Lys Gly Glu Arg Met Ala Asp Gly Ala
            105                 110                 115

ccc ctg gct gga gtg aga ggg ggc tta agt gat ggg gag ggt ccc cct      798
Pro Leu Ala Gly Val Arg Gly Gly Leu Ser Asp Gly Glu Gly Pro Pro
        120                 125                 130

ggg ggt cgc ggg gag gcg cag cgg cgt aaa gat cgg gaa gaa ttg gct      846
Gly Gly Arg Gly Glu Ala Gln Arg Arg Lys Asp Arg Glu Glu Leu Ala
    135                 140                 145

cag cag tat gag acc atc ctc cgg gag tgc ggc cat ggt cgc ttc cag      894
Gln Gln Tyr Glu Thr Ile Leu Arg Glu Cys Gly His Gly Arg Phe Gln
150                 155                 160                 165

tgg aca ctc tac ttc gtg ctg ggt ctg gcg ctg atg gcc gat ggt gta      942
Trp Thr Leu Tyr Phe Val Leu Gly Leu Ala Leu Met Ala Asp Gly Val
                170                 175                 180

gag gtc ttt gtg gtg ggc ttt gtg ctg ccc agt gct gag aaa gat atg      990
Glu Val Phe Val Val Gly Phe Val Leu Pro Ser Ala Glu Lys Asp Met
            185                 190                 195

tgc ctg tcg gac tcc aac aaa ggc atg cta ggc ctc att gtg tac ctg     1038
Cys Leu Ser Asp Ser Asn Lys Gly Met Leu Gly Leu Ile Val Tyr Leu
        200                 205                 210

ggc atg atg gtg ggg gcc ttc ctc tgg gga ggc ctg gct gat cgg ctg     1086
Gly Met Met Val Gly Ala Phe Leu Trp Gly Gly Leu Ala Asp Arg Leu
    215                 220                 225

ggt cgg aga cag tgt ctg ctc atc tcg ctc tca gtc aac agc gtc ttc     1134
Gly Arg Arg Gln Cys Leu Leu Ile Ser Leu Ser Val Asn Ser Val Phe
230                 235                 240                 245

gct ttc ttc tca tcc ttc gtc cag ggt tat ggc acc ttc ctt ttc tgc     1182
Ala Phe Phe Ser Ser Phe Val Gln Gly Tyr Gly Thr Phe Leu Phe Cys
                250                 255                 260

cgc ctc ctt tct ggg gtt ggg att ggt ggt tcc atc ccc att gtc ttc     1230
Arg Leu Leu Ser Gly Val Gly Ile Gly Gly Ser Ile Pro Ile Val Phe
            265                 270                 275

tcc tat ttt tcg gag ttt ctg gcc cag gag aaa cgt ggg gag cat ttg     1278
Ser Tyr Phe Ser Glu Phe Leu Ala Gln Glu Lys Arg Gly Glu His Leu
        280                 285                 290

agc tgg ctc tgt atg ttc tgg atg att ggt ggc gtg tat gca gct gca     1326
Ser Trp Leu Cys Met Phe Trp Met Ile Gly Gly Val Tyr Ala Ala Ala
    295                 300                 305

atg gcc tgg gcc atc atc ccc cac tat ggg tgg agt ttc cag atg ggc     1374
Met Ala Trp Ala Ile Ile Pro His Tyr Gly Trp Ser Phe Gln Met Gly
310                 315                 320                 325
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tct gct tac cag ttc cac agc tgg agg gtc ttt gtc ctc gtc ttt gcc     1422
Ser Ala Tyr Gln Phe His Ser Trp Arg Val Phe Val Leu Val Phe Ala
            330                 335                 340

ttt ccc tct gtg ttt gcc atc ggg gct ctg act acg cag ccg gag agt     1470
Phe Pro Ser Val Phe Ala Ile Gly Ala Leu Thr Thr Gln Pro Glu Ser
        345                 350                 355

ccc cgc ttc ttc tta gag aat ggg aag cac gat gag gcc tgg atg gtg     1518
Pro Arg Phe Phe Leu Glu Asn Gly Lys His Asp Glu Ala Trp Met Val
    360                 365                 370

ctg aag cag gtt cat gac acc aac atg cga gcc aag ggc cat cct gag     1566
Leu Lys Gln Val His Asp Thr Asn Met Arg Ala Lys Gly His Pro Glu
375                 380                 385

cga gtc ttc tca gta acc cac att aaa acg att cat cag gag gat gaa     1614
Arg Val Phe Ser Val Thr His Ile Lys Thr Ile His Gln Glu Asp Glu
390                 395                 400                 405

ttg att gag atc cag tca gac aca gga acc tgg tac cag cgc tgg gga     1662
Leu Ile Glu Ile Gln Ser Asp Thr Gly Thr Trp Tyr Gln Arg Trp Gly
            410                 415                 420

gtg cgg gct ttg agc ctg ggg ggt cag gtt tgg ggg aac ttc ctc tcc     1710
Val Arg Ala Leu Ser Leu Gly Gly Gln Val Trp Gly Asn Phe Leu Ser
        425                 430                 435

tgc ttc agt cca gag tac cgg cgc atc act ctg atg atg atg ggg gta     1758
Cys Phe Ser Pro Glu Tyr Arg Arg Ile Thr Leu Met Met Met Gly Val
    440                 445                 450

tgg ttc acc atg tcc ttc agc tac tac ggt ttg act gtc tgg ttt ccc     1806
Trp Phe Thr Met Ser Phe Ser Tyr Tyr Gly Leu Thr Val Trp Phe Pro
455                 460                 465

gac atg atc cgc cat ctc cag gct gtg gac tat gca gcc cga acc aaa     1854
Asp Met Ile Arg His Leu Gln Ala Val Asp Tyr Ala Ala Arg Thr Lys
470                 475                 480                 485

gtg ttc cca ggg gag cgc gtg gag cac gtg aca ttt aac ttc aca ctg     1902
Val Phe Pro Gly Glu Arg Val Glu His Val Thr Phe Asn Phe Thr Leu
            490                 495                 500

gag aat cag atc cac cga ggg gga cag tac ttc aat gac aag ttc atc     1950
Glu Asn Gln Ile His Arg Gly Gly Gln Tyr Phe Asn Asp Lys Phe Ile
        505                 510                 515

ggg ctg cgt ctg aag tca gtg tcc ttt gag gat tcc ctg ttt gag gaa     1998
Gly Leu Arg Leu Lys Ser Val Ser Phe Glu Asp Ser Leu Phe Glu Glu
    520                 525                 530

tgt tac ttt gaa gat gtc aca tcc agc aac aca ttc ttc cgc aac tgc     2046
Cys Tyr Phe Glu Asp Val Thr Ser Ser Asn Thr Phe Phe Arg Asn Cys
535                 540                 545

aca ttc atc aac acc gtg ttc tac aac acg gac ctg ttt gag tac aag     2094
Thr Phe Ile Asn Thr Val Phe Tyr Asn Thr Asp Leu Phe Glu Tyr Lys
550                 555                 560                 565

ttc gtg aac agc cgc ctg gtg aac agc aca ttc ctg cac aat aag gaa     2142
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Phe Val Asn Ser Arg Leu Val Asn Ser Thr Phe Leu His Asn Lys Glu
                570                 575                 580

ggt tgc cca cta gat gtg aca ggg acg ggc gaa ggt gcc tac atg gtg     2190
Gly Cys Pro Leu Asp Val Thr Gly Thr Gly Glu Gly Ala Tyr Met Val
            585                 590                 595

tac ttt gtc agc ttc ttg ggg aca ctg gct gtg ctc cct gga aat att     2238
Tyr Phe Val Ser Phe Leu Gly Thr Leu Ala Val Leu Pro Gly Asn Ile
        600                 605                 610

gtg tct gct ctg ctc atg gac aag att ggc agg ctc aga atg ctt gct     2286
Val Ser Ala Leu Leu Met Asp Lys Ile Gly Arg Leu Arg Met Leu Ala
    615                 620                 625

ggt tcc agt gtg ttg tcc tgt gtt tcc tgc ttc ttc ctg tct ttt ggg     2334
Gly Ser Ser Val Leu Ser Cys Val Ser Cys Phe Phe Leu Ser Phe Gly
630                 635                 640                 645

aac agt gag tca gcc atg atc gct ctg ctc tgc ctt ttt ggg gga gtc     2382
Asn Ser Glu Ser Ala Met Ile Ala Leu Leu Cys Leu Phe Gly Gly Val
                650                 655                 660

agt att gca tcc tgg aac gcg ctg gac gtg ctg act gtt gaa ctc tac     2430
Ser Ile Ala Ser Trp Asn Ala Leu Asp Val Leu Thr Val Glu Leu Tyr
            665                 670                 675

cct tcc gac aag agg acg acg gcc ttc ggc ttc ctg aat gcc ctg tgt     2478
Pro Ser Asp Lys Arg Thr Thr Ala Phe Gly Phe Leu Asn Ala Leu Cys
        680                 685                 690

aag ctg gca gct gta ctg ggc atc agc atc ttc acg tcc ttt gtg gga     2526
Lys Leu Ala Ala Val Leu Gly Ile Ser Ile Phe Thr Ser Phe Val Gly
    695                 700                 705

atc acc aag gcc gct ccc atc ctc ttc gcc tca gct gcg ctt gcc ctt     2574
Ile Thr Lys Ala Ala Pro Ile Leu Phe Ala Ser Ala Ala Leu Ala Leu
710                 715                 720                 725

ggt agc tct ctg gct ctg aag ctg cct gag acc cgg gga cag gtg ctg     2622
Gly Ser Ser Leu Ala Leu Lys Leu Pro Glu Thr Arg Gly Gln Val Leu
                730                 735                 740

cag tga gggatggggg agtgtctcag gggctttagg gatggcaggc acactgtgac      2678
Gln

caataatttc ttttatccct accctgccct gctgtcctgg tcctactccg tgtttggtgt   2738

cttagctgtg tgcctgtgtg catgtgtgtg accctgacgg gcaggggcta cggggagggt   2798

ccctttgtcc catgtttggg aggagggact ccccacctgc tgccaccctc aactttgcac   2858

aaggagaagg ctgagctgca tccttctctc cctcagtgtt agcaggggtg gggacgactg   2918

tttctctgct ccaggtgttc cagaatttct gcctttccca tcattccctc cgcctaggcc   2978

ctggtgaacc acaggtatgg agttatagtg ggggctgagg cttggaccaa aagaacttct   3038

tgagtgggag cctcccaagg atgctgggga gtagcaataa accttagcct ccgttttcac   3098

ctcaattcag gctacaagtg tgaagcctgg attttatgga attagttttc tgattcttat   3158
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atgtgtgtgt gtgtgcgccg cgtgcgtgtg cgtgtgtgta tgtgtgccat ggggtagggg 3278

taccactata ctgttcaatt ataagccaag agtagtagtt tcagtgagca cacacacaac 3338

actgtttttc tatcgtaact cccagaatct tgtacctgtg ttggggctgc aggcagaagt 3398

ccttggtcag gctggggtga gccctgcaat cttgggggac ctgagggcac ctgacaagga 3458

ctttctcctt cctctagaga ggttctaccc actagccaca gccctcccat ctgacctgtc 3518

cacacaggca gtgtatcaga ggaaagaaag ggaaaataac caggcacaca tggtcaaacc 3578

agcaggtctg aaagcacaag aagctggggc aggggcagga agcaggggtc catcccctaa 3638

cccttctcaa aaaggctggg tcgtgaggga cccctaatgc agggacctga agcctcagtt 3698

tccccatctt gcccttccac agaacagcct ctgtaggtag agctgccccc cgtcctaccc 3758

tactcttgtg gctgctttct ttggtactct tcccactccc accgtagctg tgacgtgttg 3818

tagtttttag ctgtttgtaa aatgtt 3844

<210> 10
<211> 742
<212> PRT
<213> Rattus norvegicus

<400> 10

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Met Glu Glu Gly Phe Arg Asp Arg Ala Ala Phe Ile Arg Gly Ala Lys
1               5                  10                  15

Asp Ile Ala Lys Glu Val Lys Lys His Ala Ala Lys Lys Val Val Lys
            20                  25                  30

Gly Leu Asp Arg Val Gln Asp Glu Tyr Ser Arg Arg Ser Tyr Ser Arg
        35                  40                  45

Phe Glu Glu Glu Glu Asp Asp Asp Asp Phe Pro Ala Pro Ala Asp Gly
    50                  55                  60

Tyr Tyr Arg Gly Glu Gly Ala Gln Asp Glu Glu Glu Gly Gly Ala Ser
65                  70                  75                  80

Ser Asp Ala Thr Glu Gly His Asp Glu Asp Asp Glu Ile Tyr Glu Gly
                85                  90                  95

Glu Tyr Gln Gly Ile Pro Arg Ala Glu Ser Gly Gly Lys Gly Glu Arg
            100                 105                 110

Met Ala Asp Gly Ala Pro Leu Ala Gly Val Arg Gly Gly Leu Ser Asp
        115                 120                 125

Gly Glu Gly Pro Pro Gly Gly Arg Gly Glu Ala Gln Arg Arg Lys Asp
    130                 135                 140

Arg Glu Glu Leu Ala Gln Gln Tyr Glu Thr Ile Leu Arg Glu Cys Gly
145                 150                 155                 160
```

```
His Gly Arg Phe Gln Trp Thr Leu Tyr Phe Val Leu Gly Leu Ala Leu
                165                 170                 175

Met Ala Asp Gly Val Glu Val Phe Val Val Gly Phe Val Leu Pro Ser
            180                 185                 190

Ala Glu Lys Asp Met Cys Leu Ser Asp Ser Asn Lys Gly Met Leu Gly
        195                 200                 205

Leu Ile Val Tyr Leu Gly Met Met Val Gly Ala Phe Leu Trp Gly Gly
    210                 215                 220

Leu Ala Asp Arg Leu Gly Arg Arg Gln Cys Leu Leu Ile Ser Leu Ser
225                 230                 235                 240

Val Asn Ser Val Phe Ala Phe Phe Ser Ser Phe Val Gln Gly Tyr Gly
                245                 250                 255

Thr Phe Leu Phe Cys Arg Leu Leu Ser Gly Val Gly Ile Gly Gly Ser
            260                 265                 270

Ile Pro Ile Val Phe Ser Tyr Phe Ser Glu Phe Leu Ala Gln Glu Lys
        275                 280                 285

Arg Gly Glu His Leu Ser Trp Leu Cys Met Phe Trp Met Ile Gly Gly
    290                 295                 300

Val Tyr Ala Ala Ala Met Ala Trp Ala Ile Ile Pro His Tyr Gly Trp
305                 310                 315                 320

Ser Phe Gln Met Gly Ser Ala Tyr Gln Phe His Ser Trp Arg Val Phe
                325                 330                 335

Val Leu Val Phe Ala Phe Pro Ser Val Phe Ala Ile Gly Ala Leu Thr
            340                 345                 350

Thr Gln Pro Glu Ser Pro Arg Phe Phe Leu Glu Asn Gly Lys His Asp
        355                 360                 365

Glu Ala Trp Met Val Leu Lys Gln Val His Asp Thr Asn Met Arg Ala
    370                 375                 380

Lys Gly His Pro Glu Arg Val Phe Ser Val Thr His Ile Lys Thr Ile
385                 390                 395                 400

His Gln Glu Asp Glu Leu Ile Glu Ile Gln Ser Asp Thr Gly Thr Trp
                405                 410                 415

Tyr Gln Arg Trp Gly Val Arg Ala Leu Ser Leu Gly Gly Gln Val Trp
            420                 425                 430

Gly Asn Phe Leu Ser Cys Phe Ser Pro Glu Tyr Arg Arg Ile Thr Leu
        435                 440                 445

Met Met Met Gly Val Trp Phe Thr Met Ser Phe Ser Tyr Tyr Gly Leu
    450                 455                 460

Thr Val Trp Phe Pro Asp Met Ile Arg His Leu Gln Ala Val Asp Tyr
465                 470                 475                 480
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```
Ala Ala Arg Thr Lys Val Phe Pro Gly Glu Arg Val Glu His Val Thr
                485                 490                 495

Phe Asn Phe Thr Leu Glu Asn Gln Ile His Arg Gly Gly Gln Tyr Phe
            500                 505                 510

Asn Asp Lys Phe Ile Gly Leu Arg Leu Lys Ser Val Ser Phe Glu Asp
        515                 520                 525

Ser Leu Phe Glu Glu Cys Tyr Phe Glu Asp Val Thr Ser Ser Asn Thr
    530                 535                 540

Phe Phe Arg Asn Cys Thr Phe Ile Asn Thr Val Phe Tyr Asn Thr Asp
545                 550                 555                 560

Leu Phe Glu Tyr Lys Phe Val Asn Ser Arg Leu Val Asn Ser Thr Phe
                565                 570                 575

Leu His Asn Lys Glu Gly Cys Pro Leu Asp Val Thr Gly Thr Gly Glu
            580                 585                 590

Gly Ala Tyr Met Val Tyr Phe Val Ser Phe Leu Gly Thr Leu Ala Val
        595                 600                 605

Leu Pro Gly Asn Ile Val Ser Ala Leu Leu Met Asp Lys Ile Gly Arg
    610                 615                 620

Leu Arg Met Leu Ala Gly Ser Ser Val Leu Ser Cys Val Ser Cys Phe
625                 630                 635                 640

Phe Leu Ser Phe Gly Asn Ser Glu Ser Ala Met Ile Ala Leu Leu Cys
                645                 650                 655

Leu Phe Gly Gly Val Ser Ile Ala Ser Trp Asn Ala Leu Asp Val Leu
            660                 665                 670

Thr Val Glu Leu Tyr Pro Ser Asp Lys Arg Thr Thr Ala Phe Gly Phe
        675                 680                 685

Leu Asn Ala Leu Cys Lys Leu Ala Ala Val Leu Gly Ile Ser Ile Phe
    690                 695                 700

Thr Ser Phe Val Gly Ile Thr Lys Ala Ala Pro Ile Leu Phe Ala Ser
705                 710                 715                 720

Ala Ala Leu Ala Leu Gly Ser Ser Leu Ala Leu Lys Leu Pro Glu Thr
                725                 730                 735

Arg Gly Gln Val Leu Gln
            740
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<210> 11
<211> 2052
<212> DNA
<213> Rattus norvegicus

<220>
<221> CDS
<222> (1)..(2052)
<223>

<400> 11

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atg gat gac tac agg tat cgg gac aac tat gag ggc tat gcc cct aat      48
Met Asp Asp Tyr Arg Tyr Arg Asp Asn Tyr Glu Gly Tyr Ala Pro Asn
1               5                  10                  15

gat ggc tac tac cgg ggc aat gag cag aac ccg gaa gaa gat gca cag      96
Asp Gly Tyr Tyr Arg Gly Asn Glu Gln Asn Pro Glu Glu Asp Ala Gln
            20                  25                  30

agc gat gtt aca gaa ggc cac gat gaa gag gat gag atc tat gag ggc     144
Ser Asp Val Thr Glu Gly His Asp Glu Glu Asp Glu Ile Tyr Glu Gly
        35                  40                  45

gag tac caa ggc atc cct cat cca gat gat gtc aag tct aag cag act     192
Glu Tyr Gln Gly Ile Pro His Pro Asp Asp Val Lys Ser Lys Gln Thr
    50                  55                  60

aag atg gca ccg tcc aga gca gat ggc ctt cgg ggc cag gca gac ctg     240
Lys Met Ala Pro Ser Arg Ala Asp Gly Leu Arg Gly Gln Ala Asp Leu
65                  70                  75                  80

atg gct gag aga atg gaa gat gag gag cag ctc gct cac cag tac gag     288
Met Ala Glu Arg Met Glu Asp Glu Glu Gln Leu Ala His Gln Tyr Glu
                85                  90                  95

acc atc att gat gag tgt ggc cat ggg cgc ttc cag tgg acc ctc ttt     336
Thr Ile Ile Asp Glu Cys Gly His Gly Arg Phe Gln Trp Thr Leu Phe
            100                 105                 110

ttc gtc ttg gtc ttg gcc ttg atg gct gac gga gtg gaa gtg ttt gtg     384
Phe Val Leu Val Leu Ala Leu Met Ala Asp Gly Val Glu Val Phe Val
        115                 120                 125

gtg agc ttt gct ctg cca agt gca gag aaa gat atg tgt ctg tca agt     432
Val Ser Phe Ala Leu Pro Ser Ala Glu Lys Asp Met Cys Leu Ser Ser
    130                 135                 140

tcc aag aaa gga atg ctc ggg ctg att gtc tac cta gga atg atg gca     480
Ser Lys Lys Gly Met Leu Gly Leu Ile Val Tyr Leu Gly Met Met Ala
145                 150                 155                 160

gga gcc ttc atc ctg ggg ggc ctg gct gat aaa ctg gga agg aag aag     528
Gly Ala Phe Ile Leu Gly Gly Leu Ala Asp Lys Leu Gly Arg Lys Lys
                165                 170                 175

gtc ctc agc atg tcc ttg gct atc aat gct tcc ttt gcc tcc ctc tcc     576
Val Leu Ser Met Ser Leu Ala Ile Asn Ala Ser Phe Ala Ser Leu Ser
            180                 185                 190

tcc ttc gtg cag gga tat gga gct ttc ctc ttc tgc aga ctc atc tca     624
Ser Phe Val Gln Gly Tyr Gly Ala Phe Leu Phe Cys Arg Leu Ile Ser
        195                 200                 205

ggc ata ggt att ggg ggc tcc ctg cca att gtt ttt gcc tac ttt tct     672
Gly Ile Gly Ile Gly Gly Ser Leu Pro Ile Val Phe Ala Tyr Phe Ser
    210                 215                 220

gag ttc tta tca cgg gag aaa cgc ggt gag cat ctc agc tgg ctg ggt     720
Glu Phe Leu Ser Arg Glu Lys Arg Gly Glu His Leu Ser Trp Leu Gly
225                 230                 235                 240
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atc ttc tgg atg act ggg ggc atc tac gca tct gcc atg gcc tgg agc      768
Ile Phe Trp Met Thr Gly Gly Ile Tyr Ala Ser Ala Met Ala Trp Ser
                245                 250                 255

atc att cca cac tat ggc tgg ggc ttc agc atg gga acc aat tat cac      816
Ile Ile Pro His Tyr Gly Trp Gly Phe Ser Met Gly Thr Asn Tyr His
            260                 265                 270

ttc cac agc tgg aga gtg ttt gtc atc gtc tgt gct ctg cct gcc act      864
Phe His Ser Trp Arg Val Phe Val Ile Val Cys Ala Leu Pro Ala Thr
        275                 280                 285

gtg tcc atg gtg gcc ctg aag ttc atg cca gaa agc ccc agg ttc ctg      912
Val Ser Met Val Ala Leu Lys Phe Met Pro Glu Ser Pro Arg Phe Leu
    290                 295                 300

ctg gag atg ggc aag cat gat gaa gcc tgg atg att ctc aag caa gtc      960
Leu Glu Met Gly Lys His Asp Glu Ala Trp Met Ile Leu Lys Gln Val
305                 310                 315                 320

cat gac acc aac atg aga gct aag ggg acc cct gag aag gtg ttc acg     1008
His Asp Thr Asn Met Arg Ala Lys Gly Thr Pro Glu Lys Val Phe Thr
                325                 330                 335

gtt tcc cac atc aaa act ccc aag caa atg gat gaa ttc att gag atc     1056
Val Ser His Ile Lys Thr Pro Lys Gln Met Asp Glu Phe Ile Glu Ile
            340                 345                 350

cag agt tca aca ggg act tgg tac cag cgc tgg ttg gtc agg ttc atg     1104
Gln Ser Ser Thr Gly Thr Trp Tyr Gln Arg Trp Leu Val Arg Phe Met
        355                 360                 365

acc att ttc aaa cag gtg tgg gat aac gcc ttg tac tgt gtg atg gga     1152
Thr Ile Phe Lys Gln Val Trp Asp Asn Ala Leu Tyr Cys Val Met Gly
    370                 375                 380

ccc tac aga atg aac acc ctg att ctg gct gtg gtc tgg ttc acc atg     1200
Pro Tyr Arg Met Asn Thr Leu Ile Leu Ala Val Val Trp Phe Thr Met
385                 390                 395                 400

gcc tta agt tac tat ggc ctg aca gtg tgg ttc ccc gac atg atc cgg     1248
Ala Leu Ser Tyr Tyr Gly Leu Thr Val Trp Phe Pro Asp Met Ile Arg
                405                 410                 415

tat ttc cag gat gaa gaa tat aag tct aaa atg aag gtg ttt ttt ggt     1296
Tyr Phe Gln Asp Glu Glu Tyr Lys Ser Lys Met Lys Val Phe Phe Gly
            420                 425                 430

gag cac gtg cat ggc gcc aca atc aac ttc acc atg gaa aac cag atc     1344
Glu His Val His Gly Ala Thr Ile Asn Phe Thr Met Glu Asn Gln Ile
        435                 440                 445

cac caa cat ggg aag ctt gtg aac gat aag ttc ata aag atg tac ttt     1392
His Gln His Gly Lys Leu Val Asn Asp Lys Phe Ile Lys Met Tyr Phe
    450                 455                 460

aag cat gtc ctc ttt gag gac aca ttc ttt gac aaa tgc tat ttt gaa     1440
Lys His Val Leu Phe Glu Asp Thr Phe Phe Asp Lys Cys Tyr Phe Glu
465                 470                 475                 480
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gat gtg aca tcc aca gat act tat ttc aag aac tgc acc att gaa tcg     1488
Asp Val Thr Ser Thr Asp Thr Tyr Phe Lys Asn Cys Thr Ile Glu Ser
                485                 490                 495

act acc ttc tac aac aca gac ctc tac aaa cac aag ttc att gac tgt     1536
Thr Thr Phe Tyr Asn Thr Asp Leu Tyr Lys His Lys Phe Ile Asp Cys
            500                 505                 510

cgg ttt atc aat tcc acc ttt ctg gag cag aag gag ggc tgc cac atg     1584
Arg Phe Ile Asn Ser Thr Phe Leu Glu Gln Lys Glu Gly Cys His Met
        515                 520                 525

gac ttt gaa gag gac aat gat ttt ctg att tac ctc gtc agc ttc ctc     1632
Asp Phe Glu Glu Asp Asn Asp Phe Leu Ile Tyr Leu Val Ser Phe Leu
    530                 535                 540

ggc agc ctg tct gtc ttg cct ggg aac ata att tct gcc ctg ctc atg     1680
Gly Ser Leu Ser Val Leu Pro Gly Asn Ile Ile Ser Ala Leu Leu Met
545                 550                 555                 560

gac aga atc gga aga ctt aag atg att ggt ggc tcc atg ctc atc tct     1728
Asp Arg Ile Gly Arg Leu Lys Met Ile Gly Gly Ser Met Leu Ile Ser
                565                 570                 575

gca gtc tgc tgc ttc ttc ctg ttt ttt ggc aac agc gag tct gcg atg     1776
Ala Val Cys Cys Phe Phe Leu Phe Phe Gly Asn Ser Glu Ser Ala Met
            580                 585                 590

atc ggc tgg caa tgc ctg ttc tgt ggg acc agc att gca gcc tgg aat     1824
Ile Gly Trp Gln Cys Leu Phe Cys Gly Thr Ser Ile Ala Ala Trp Asn
        595                 600                 605

gct ctg gat gtg atc aca gta gag ctg tat ccc acc aac cag agg gcc     1872
Ala Leu Asp Val Ile Thr Val Glu Leu Tyr Pro Thr Asn Gln Arg Ala
    610                 615                 620

act gcc ttc ggc atc ctc aat gga ctg tgc aaa ctt ggt gcc atc ctg     1920
Thr Ala Phe Gly Ile Leu Asn Gly Leu Cys Lys Leu Gly Ala Ile Leu
625                 630                 635                 640

gga aac act atc ttt gct tct ttt gtt ggg atc acc aaa gtg gtc ccc     1968
Gly Asn Thr Ile Phe Ala Ser Phe Val Gly Ile Thr Lys Val Val Pro
                645                 650                 655

atc ctt ctg gct gct gct tct ctg gtt gga ggt ggc ttg gtt gcc ctt     2016
Ile Leu Leu Ala Ala Ala Ser Leu Val Gly Gly Gly Leu Val Ala Leu
            660                 665                 670

cga ctg cca gag act cga gag cag gtc ctg atg tga                    2052
Arg Leu Pro Glu Thr Arg Glu Gln Val Leu Met
        675                 680
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<210> 12
<211> 683
<212> PRT
<213> Rattus norvegicus

<400> 12

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Met Asp Asp Tyr Arg Tyr Arg Asp Asn Tyr Glu Gly Tyr Ala Pro Asn
1               5                   10                  15
```

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Asp Gly Tyr Tyr Arg Gly Asn Glu Gln Asn Pro Glu Glu Asp Ala Gln
            20                  25                  30

Ser Asp Val Thr Glu Gly His Asp Glu Glu Asp Glu Ile Tyr Glu Gly
        35                  40                  45

Glu Tyr Gln Gly Ile Pro His Pro Asp Asp Val Lys Ser Lys Gln Thr
    50                  55                  60

Lys Met Ala Pro Ser Arg Ala Asp Gly Leu Arg Gly Gln Ala Asp Leu
65                  70                  75                  80

Met Ala Glu Arg Met Glu Asp Glu Glu Gln Leu Ala His Gln Tyr Glu
                85                  90                  95

Thr Ile Ile Asp Glu Cys Gly His Gly Arg Phe Gln Trp Thr Leu Phe
            100                 105                 110

Phe Val Leu Val Leu Ala Leu Met Ala Asp Gly Val Glu Val Phe Val
        115                 120                 125

Val Ser Phe Ala Leu Pro Ser Ala Glu Lys Asp Met Cys Leu Ser Ser
    130                 135                 140

Ser Lys Lys Gly Met Leu Gly Leu Ile Val Tyr Leu Gly Met Met Ala
145                 150                 155                 160

Gly Ala Phe Ile Leu Gly Gly Leu Ala Asp Lys Leu Gly Arg Lys Lys
                165                 170                 175

Val Leu Ser Met Ser Leu Ala Ile Asn Ala Ser Phe Ala Ser Leu Ser
            180                 185                 190

Ser Phe Val Gln Gly Tyr Gly Ala Phe Leu Phe Cys Arg Leu Ile Ser
        195                 200                 205

Gly Ile Gly Ile Gly Gly Ser Leu Pro Ile Val Phe Ala Tyr Phe Ser
    210                 215                 220

Glu Phe Leu Ser Arg Glu Lys Arg Gly Glu His Leu Ser Trp Leu Gly
225                 230                 235                 240

Ile Phe Trp Met Thr Gly Gly Ile Tyr Ala Ser Ala Met Ala Trp Ser
                245                 250                 255

Ile Ile Pro His Tyr Gly Trp Gly Phe Ser Met Gly Thr Asn Tyr His
            260                 265                 270

Phe His Ser Trp Arg Val Phe Val Ile Val Cys Ala Leu Pro Ala Thr
        275                 280                 285

Val Ser Met Val Ala Leu Lys Phe Met Pro Glu Ser Pro Arg Phe Leu
    290                 295                 300

Leu Glu Met Gly Lys His Asp Glu Ala Trp Met Ile Leu Lys Gln Val
305                 310                 315                 320

His Asp Thr Asn Met Arg Ala Lys Gly Thr Pro Glu Lys Val Phe Thr
                325                 330                 335
```

```
Val Ser His Ile Lys Thr Pro Lys Gln Met Asp Glu Phe Ile Glu Ile
            340                 345                 350

Gln Ser Ser Thr Gly Thr Trp Tyr Gln Arg Trp Leu Val Arg Phe Met
        355                 360                 365

Thr Ile Phe Lys Gln Val Trp Asp Asn Ala Leu Tyr Cys Val Met Gly
    370                 375                 380

Pro Tyr Arg Met Asn Thr Leu Ile Leu Ala Val Val Trp Phe Thr Met
385                 390                 395                 400

Ala Leu Ser Tyr Tyr Gly Leu Thr Val Trp Phe Pro Asp Met Ile Arg
                405                 410                 415

Tyr Phe Gln Asp Glu Glu Tyr Lys Ser Lys Met Lys Val Phe Phe Gly
            420                 425                 430

Glu His Val His Gly Ala Thr Ile Asn Phe Thr Met Glu Asn Gln Ile
        435                 440                 445

His Gln His Gly Lys Leu Val Asn Asp Lys Phe Ile Lys Met Tyr Phe
    450                 455                 460

Lys His Val Leu Phe Glu Asp Thr Phe Phe Asp Lys Cys Tyr Phe Glu
465                 470                 475                 480

Asp Val Thr Ser Thr Asp Thr Tyr Phe Lys Asn Cys Thr Ile Glu Ser
                485                 490                 495

Thr Thr Phe Tyr Asn Thr Asp Leu Tyr Lys His Lys Phe Ile Asp Cys
            500                 505                 510

Arg Phe Ile Asn Ser Thr Phe Leu Glu Gln Lys Glu Gly Cys His Met
        515                 520                 525

Asp Phe Glu Glu Asp Asn Asp Phe Leu Ile Tyr Leu Val Ser Phe Leu
    530                 535                 540

Gly Ser Leu Ser Val Leu Pro Gly Asn Ile Ile Ser Ala Leu Leu Met
545                 550                 555                 560

Asp Arg Ile Gly Arg Leu Lys Met Ile Gly Gly Ser Met Leu Ile Ser
                565                 570                 575

Ala Val Cys Cys Phe Phe Leu Phe Phe Gly Asn Ser Glu Ser Ala Met
            580                 585                 590

Ile Gly Trp Gln Cys Leu Phe Cys Gly Thr Ser Ile Ala Ala Trp Asn
        595                 600                 605

Ala Leu Asp Val Ile Thr Val Glu Leu Tyr Pro Thr Asn Gln Arg Ala
    610                 615                 620

Thr Ala Phe Gly Ile Leu Asn Gly Leu Cys Lys Leu Gly Ala Ile Leu
625                 630                 635                 640

Gly Asn Thr Ile Phe Ala Ser Phe Val Gly Ile Thr Lys Val Val Pro
                645                 650                 655

Ile Leu Leu Ala Ala Ala Ser Leu Val Gly Gly Gly Leu Val Ala Leu
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660 665 670

Arg Leu Pro Glu Thr Arg Glu Gln Val Leu Met
675 680

<210> 13
<211> 2622
<212> DNA
<213> Rattus norvegicus

<220>
<221> CDS
<222> (224)..(2407)
<223>

<400> 13
gcgcgctgca ggaagagtgg cagaccgaag cggcctcggg ctgcaaacgg aggggcgctc 60

gcgcggcgac ggctgcaggg ctgacaccgc tcagggcagg ggggtcccag gcggctggaa 120

cgctctattc tgaactgtga gtggatgatg ctgttgcagc caagctgctg aacacactcc 180

gtggactctt ccctgctgtg ccttgcccat cggccgagat aaa atg gaa gac tcc 235
Met Glu Asp Ser
1

tac aag gat agg act tca ctg atg aag ggc gcc aag gac att gcc aaa 283
Tyr Lys Asp Arg Thr Ser Leu Met Lys Gly Ala Lys Asp Ile Ala Lys
5 10 15 20

gag gtg aag aag caa aca gtg aag aag gtg aac cag gca gtg gac cgg 331
Glu Val Lys Lys Gln Thr Val Lys Lys Val Asn Gln Ala Val Asp Arg
25 30 35

gcc cag gat gaa tac acc cag cgg tcc tac agt cga ttc cag gat gaa 379
Ala Gln Asp Glu Tyr Thr Gln Arg Ser Tyr Ser Arg Phe Gln Asp Glu
40 45 50

gat gat gat gat gac tac tac cca cct gga gaa acc tac agt ggg gag 427
Asp Asp Asp Asp Asp Tyr Tyr Pro Pro Gly Glu Thr Tyr Ser Gly Glu
55 60 65

gcc aat gat gat gaa ggc tca agt gaa gcc act gag ggt cac gat gaa 475
Ala Asn Asp Asp Glu Gly Ser Ser Glu Ala Thr Glu Gly His Asp Glu
70 75 80

gaa gac gag atc tat gaa ggg gaa tac cag ggc atc ccc agc acg aac 523
Glu Asp Glu Ile Tyr Glu Gly Glu Tyr Gln Gly Ile Pro Ser Thr Asn
85 90 95 100

caa ggg aag gac agc ata gtg tct gta gga caa ccc aaa gga gat gag 571
Gln Gly Lys Asp Ser Ile Val Ser Val Gly Gln Pro Lys Gly Asp Glu
105 110 115

tac aag gac cgc aga gag ctg gag tca gag agg agg gct gat gag gag 619
Tyr Lys Asp Arg Arg Glu Leu Glu Ser Glu Arg Arg Ala Asp Glu Glu
120 125 130

gag ctc gcc cag cag tat gag ctg ata atc caa gag tgt ggc cat ggc 667
Glu Leu Ala Gln Gln Tyr Glu Leu Ile Ile Gln Glu Cys Gly His Gly

```
        135                 140                 145

cgt ttc cag tgg gcc ctt ttc ttc gtc ctg ggc atg gct ctc atg gca      715
Arg Phe Gln Trp Ala Leu Phe Phe Val Leu Gly Met Ala Leu Met Ala
    150                 155                 160

gac ggc gtg gag gtg ttt gtg gtg ggc ttt gtg tta ccc agt gca gag      763
Asp Gly Val Glu Val Phe Val Val Gly Phe Val Leu Pro Ser Ala Glu
165                 170                 175                 180

aca gac cta tgc ata ccg aat tca gga tct gga tgg cta ggc agc ata      811
Thr Asp Leu Cys Ile Pro Asn Ser Gly Ser Gly Trp Leu Gly Ser Ile
                185                 190                 195

gtg tac ctc ggg atg atg gtg ggg gcg ttc ttc tgg gga gga ctg gca      859
Val Tyr Leu Gly Met Met Val Gly Ala Phe Phe Trp Gly Gly Leu Ala
            200                 205                 210

gac aaa gtg gga agg aag cag tct ctt ctg att tgc atg tcc gtc aac      907
Asp Lys Val Gly Arg Lys Gln Ser Leu Leu Ile Cys Met Ser Val Asn
        215                 220                 225

gga ttc ttt gcc ttc ctt tct tca ttt gtc caa ggt tac ggc ttc ttt      955
Gly Phe Phe Ala Phe Leu Ser Ser Phe Val Gln Gly Tyr Gly Phe Phe
    230                 235                 240

ctc ctc tgt cgt ttg ctt tca gga ttc ggg att gga ggc gcc att ccc     1003
Leu Leu Cys Arg Leu Leu Ser Gly Phe Gly Ile Gly Gly Ala Ile Pro
245                 250                 255                 260

act gtg ttc tcc tac ttt gct gaa gtc ctg gcc cgg gag aag cgc ggt     1051
Thr Val Phe Ser Tyr Phe Ala Glu Val Leu Ala Arg Glu Lys Arg Gly
                265                 270                 275

gag cac ctc agt tgg ctc tgc atg ttc tgg atg att ggc ggt atc tat     1099
Glu His Leu Ser Trp Leu Cys Met Phe Trp Met Ile Gly Gly Ile Tyr
            280                 285                 290

gct tca gcc atg gcc tgg gcc atc atc ccc cac tat ggg tgg agc ttc     1147
Ala Ser Ala Met Ala Trp Ala Ile Ile Pro His Tyr Gly Trp Ser Phe
        295                 300                 305

agc atg ggc tca gcc tac cag ttc cac agc tgg cgc gtc ttc gtc atc     1195
Ser Met Gly Ser Ala Tyr Gln Phe His Ser Trp Arg Val Phe Val Ile
    310                 315                 320

gtc tgt gcc ctc ccg tgc gtc tcc tcg gtg gtg gcc ctc acc ttc atg     1243
Val Cys Ala Leu Pro Cys Val Ser Ser Val Val Ala Leu Thr Phe Met
325                 330                 335                 340

ccc gaa agc cct cgg ttc ttg ctg gag gta gga aaa cat gat gaa gcc     1291
Pro Glu Ser Pro Arg Phe Leu Leu Glu Val Gly Lys His Asp Glu Ala
                345                 350                 355

tgg atg att ctg aag cta att cat gat acc aac atg aga gcc cgg ggc     1339
Trp Met Ile Leu Lys Leu Ile His Asp Thr Asn Met Arg Ala Arg Gly
            360                 365                 370

cag cca gag aag gtc ttc acg gta aat aaa atc aag act ccc aag caa     1387
Gln Pro Glu Lys Val Phe Thr Val Asn Lys Ile Lys Thr Pro Lys Gln
        375                 380                 385
```

```
ata gat gag ctg att gag att gag agc gac aca gga acc tgg tac cgg      1435
Ile Asp Glu Leu Ile Glu Ile Glu Ser Asp Thr Gly Thr Trp Tyr Arg
    390                 395                 400

agg tgt ttt gtt cgg atc cgc aca gaa ctg tac gga att tgg ttg act      1483
Arg Cys Phe Val Arg Ile Arg Thr Glu Leu Tyr Gly Ile Trp Leu Thr
405                 410                 415                 420

ttt atg aga tgc ttc aac tac ccg gtc agg gaa aac acc ata aag ctt      1531
Phe Met Arg Cys Phe Asn Tyr Pro Val Arg Glu Asn Thr Ile Lys Leu
                425                 430                 435

acg att gtt tgg ttc acc ctg tcc ttt ggg tac tat gga ctg tcc gtt      1579
Thr Ile Val Trp Phe Thr Leu Ser Phe Gly Tyr Tyr Gly Leu Ser Val
            440                 445                 450

tgg ttc cca gat gtc att aaa cac ctc cag tct gac gag tac gcc ctg      1627
Trp Phe Pro Asp Val Ile Lys His Leu Gln Ser Asp Glu Tyr Ala Leu
        455                 460                 465

ctg act cgg aat gtg cag aag gat aaa tat gca aac ttt agc att aac      1675
Leu Thr Arg Asn Val Gln Lys Asp Lys Tyr Ala Asn Phe Ser Ile Asn
    470                 475                 480

ttc acc atg gaa aac cag gtc cac acc gga atg gaa tat gac aat ggc      1723
Phe Thr Met Glu Asn Gln Val His Thr Gly Met Glu Tyr Asp Asn Gly
485                 490                 495                 500

agg ttc ctc gga gtc aaa ttc aaa tcg gta acc ttc aag gat tca gtg      1771
Arg Phe Leu Gly Val Lys Phe Lys Ser Val Thr Phe Lys Asp Ser Val
                505                 510                 515

ttt aag tcc tgc acc ttt gac gat gtg acc tca gtc aac acc tac ttc      1819
Phe Lys Ser Cys Thr Phe Asp Asp Val Thr Ser Val Asn Thr Tyr Phe
            520                 525                 530

aag aac tgc acg ttt att gat acc ctt ttt gag aac aca gat ttt gag      1867
Lys Asn Cys Thr Phe Ile Asp Thr Leu Phe Glu Asn Thr Asp Phe Glu
        535                 540                 545

ccc tat aaa ttc ata gac agc gag ttt caa aac tgc tcg ttt ctt cac      1915
Pro Tyr Lys Phe Ile Asp Ser Glu Phe Gln Asn Cys Ser Phe Leu His
    550                 555                 560

aat aag acg gga tgt cag att act ttt gac gac gac tac agt gcc tac      1963
Asn Lys Thr Gly Cys Gln Ile Thr Phe Asp Asp Asp Tyr Ser Ala Tyr
565                 570                 575                 580

tgg att tac ttt gtc aac ttt ctc ggg aca ttg gca gtg tta cca gga      2011
Trp Ile Tyr Phe Val Asn Phe Leu Gly Thr Leu Ala Val Leu Pro Gly
                585                 590                 595

aat atc gtg tct gct ctc ctg atg gac agg atc ggg cgc tta acg atg      2059
Asn Ile Val Ser Ala Leu Leu Met Asp Arg Ile Gly Arg Leu Thr Met
            600                 605                 610

cta ggt ggc tcc atg gtg ctc tcg ggg atc agc tgc ttc ttc ctg tgg      2107
Leu Gly Gly Ser Met Val Leu Ser Gly Ile Ser Cys Phe Phe Leu Trp
        615                 620                 625
```

```
ttt ggc acc agc gaa tcc atg atg ata ggc atg ctg tgc ttg tac aac     2155
Phe Gly Thr Ser Glu Ser Met Met Ile Gly Met Leu Cys Leu Tyr Asn
    630                 635                 640

gga ctg acc atc tca gcg tgg aac tct ctt gat gta gtc acg gtg gaa     2203
Gly Leu Thr Ile Ser Ala Trp Asn Ser Leu Asp Val Val Thr Val Glu
645                 650                 655                 660

cta tac ccc aca gac cgg aga gca acg ggc ttt ggc ttc ttg aac gca     2251
Leu Tyr Pro Thr Asp Arg Arg Ala Thr Gly Phe Gly Phe Leu Asn Ala
                665                 670                 675

ctc tgt aaa gca gcg gcc gtc ctg gga aac tta ata ttc ggc tcc ttg     2299
Leu Cys Lys Ala Ala Ala Val Leu Gly Asn Leu Ile Phe Gly Ser Leu
            680                 685                 690

gtc agc atc acc aaa gca atc cct atc ctg ctg gct tcc acc gtg ctc     2347
Val Ser Ile Thr Lys Ala Ile Pro Ile Leu Leu Ala Ser Thr Val Leu
        695                 700                 705

gtg tgt gga gga ctc gtg ggg ctg cgc ctg ccc gac aca aga acc cag     2395
Val Cys Gly Gly Leu Val Gly Leu Arg Leu Pro Asp Thr Arg Thr Gln
    710                 715                 720

gtt ctg atg tga caaaagccat tctcttctct caccatgggt cagccctatt         2447
Val Leu Met
725

gcctgactca aggcttcaga gtttttatgt atagaaaggt ggccaagtat cagaactcaa   2507

acttttgctg tgacgtaaat gtagctgtgt attgtccccg ccagtgtgat ttgcagggtc   2567

ctccccctcc cccgcgcctt gttatctttt cctaattgtg atgttcctgc ttccg        2622
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<210> 14
<211> 727
<212> PRT
<213> Rattus norvegicus

<400> 14

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Met Glu Asp Ser Tyr Lys Asp Arg Thr Ser Leu Met Lys Gly Ala Lys
1               5                   10                  15

Asp Ile Ala Lys Glu Val Lys Lys Gln Thr Val Lys Lys Val Asn Gln
            20                  25                  30

Ala Val Asp Arg Ala Gln Asp Glu Tyr Thr Gln Arg Ser Tyr Ser Arg
        35                  40                  45

Phe Gln Asp Glu Asp Asp Asp Asp Asp Tyr Tyr Pro Pro Gly Glu Thr
    50                  55                  60

Tyr Ser Gly Glu Ala Asn Asp Asp Glu Gly Ser Ser Glu Ala Thr Glu
65                  70                  75                  80

Gly His Asp Glu Glu Asp Glu Ile Tyr Glu Gly Glu Tyr Gln Gly Ile
                85                  90                  95

Pro Ser Thr Asn Gln Gly Lys Asp Ser Ile Val Ser Val Gly Gln Pro
            100                 105                 110
```

```
Lys Gly Asp Glu Tyr Lys Asp Arg Arg Glu Leu Glu Ser Glu Arg Arg
        115                 120                 125

Ala Asp Glu Glu Glu Leu Ala Gln Gln Tyr Glu Leu Ile Ile Gln Glu
    130                 135                 140

Cys Gly His Gly Arg Phe Gln Trp Ala Leu Phe Phe Val Leu Gly Met
145                 150                 155                 160

Ala Leu Met Ala Asp Gly Val Glu Val Phe Val Val Gly Phe Val Leu
                165                 170                 175

Pro Ser Ala Glu Thr Asp Leu Cys Ile Pro Asn Ser Gly Ser Gly Trp
            180                 185                 190

Leu Gly Ser Ile Val Tyr Leu Gly Met Met Val Gly Ala Phe Phe Trp
        195                 200                 205

Gly Gly Leu Ala Asp Lys Val Gly Arg Lys Gln Ser Leu Leu Ile Cys
    210                 215                 220

Met Ser Val Asn Gly Phe Phe Ala Phe Leu Ser Ser Phe Val Gln Gly
225                 230                 235                 240

Tyr Gly Phe Phe Leu Leu Cys Arg Leu Leu Ser Gly Phe Gly Ile Gly
                245                 250                 255

Gly Ala Ile Pro Thr Val Phe Ser Tyr Phe Ala Glu Val Leu Ala Arg
            260                 265                 270

Glu Lys Arg Gly Glu His Leu Ser Trp Leu Cys Met Phe Trp Met Ile
        275                 280                 285

Gly Gly Ile Tyr Ala Ser Ala Met Ala Trp Ala Ile Ile Pro His Tyr
    290                 295                 300

Gly Trp Ser Phe Ser Met Gly Ser Ala Tyr Gln Phe His Ser Trp Arg
305                 310                 315                 320

Val Phe Val Ile Val Cys Ala Leu Pro Cys Val Ser Ser Val Val Ala
                325                 330                 335

Leu Thr Phe Met Pro Glu Ser Pro Arg Phe Leu Leu Glu Val Gly Lys
            340                 345                 350

His Asp Glu Ala Trp Met Ile Leu Lys Leu Ile His Asp Thr Asn Met
        355                 360                 365

Arg Ala Arg Gly Gln Pro Glu Lys Val Phe Thr Val Asn Lys Ile Lys
    370                 375                 380

Thr Pro Lys Gln Ile Asp Glu Leu Ile Glu Ile Glu Ser Asp Thr Gly
385                 390                 395                 400

Thr Trp Tyr Arg Arg Cys Phe Val Arg Ile Arg Thr Glu Leu Tyr Gly
                405                 410                 415

Ile Trp Leu Thr Phe Met Arg Cys Phe Asn Tyr Pro Val Arg Glu Asn
            420                 425                 430
```

```
Thr Ile Lys Leu Thr Ile Val Trp Phe Thr Leu Ser Phe Gly Tyr Tyr
        435                 440                 445

Gly Leu Ser Val Trp Phe Pro Asp Val Ile Lys His Leu Gln Ser Asp
    450                 455                 460
```

```
Glu Tyr Ala Leu Leu Thr Arg Asn Val Gln Lys Asp Lys Tyr Ala Asn
465                 470                 475                 480

Phe Ser Ile Asn Phe Thr Met Glu Asn Gln Val His Thr Gly Met Glu
                485                 490                 495

Tyr Asp Asn Gly Arg Phe Leu Gly Val Lys Phe Lys Ser Val Thr Phe
            500                 505                 510

Lys Asp Ser Val Phe Lys Ser Cys Thr Phe Asp Asp Val Thr Ser Val
        515                 520                 525

Asn Thr Tyr Phe Lys Asn Cys Thr Phe Ile Asp Thr Leu Phe Glu Asn
    530                 535                 540

Thr Asp Phe Glu Pro Tyr Lys Phe Ile Asp Ser Glu Phe Gln Asn Cys
545                 550                 555                 560

Ser Phe Leu His Asn Lys Thr Gly Cys Gln Ile Thr Phe Asp Asp Asp
                565                 570                 575

Tyr Ser Ala Tyr Trp Ile Tyr Phe Val Asn Phe Leu Gly Thr Leu Ala
            580                 585                 590

Val Leu Pro Gly Asn Ile Val Ser Ala Leu Leu Met Asp Arg Ile Gly
        595                 600                 605

Arg Leu Thr Met Leu Gly Gly Ser Met Val Leu Ser Gly Ile Ser Cys
    610                 615                 620

Phe Phe Leu Trp Phe Gly Thr Ser Glu Ser Met Met Ile Gly Met Leu
625                 630                 635                 640

Cys Leu Tyr Asn Gly Leu Thr Ile Ser Ala Trp Asn Ser Leu Asp Val
                645                 650                 655

Val Thr Val Glu Leu Tyr Pro Thr Asp Arg Arg Ala Thr Gly Phe Gly
            660                 665                 670

Phe Leu Asn Ala Leu Cys Lys Ala Ala Ala Val Leu Gly Asn Leu Ile
        675                 680                 685

Phe Gly Ser Leu Val Ser Ile Thr Lys Ala Ile Pro Ile Leu Leu Ala
    690                 695                 700

Ser Thr Val Leu Val Cys Gly Gly Leu Val Gly Leu Arg Leu Pro Asp
705                 710                 715                 720

Thr Arg Thr Gln Val Leu Met
                725
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<210> 15
<211> 1647
<212> DNA

<213> Rattus norvegicus

<220>
<221> CDS
<222> (1)..(1647)
<223>

<400> 15

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atg gag gag gac ctg ttc cag ctc agg cag ttg ccg gtg gtg aaa ttc       48
Met Glu Glu Asp Leu Phe Gln Leu Arg Gln Leu Pro Val Val Lys Phe
1               5                   10                  15

cgc cgc aca gga gag agc gca cgg tca gag gac gac gcg gct tcc ggg       96
Arg Arg Thr Gly Glu Ser Ala Arg Ser Glu Asp Asp Ala Ala Ser Gly
            20                  25                  30

gaa cat gat gtt cag att gag ggg gtc cga gtg ggc cta gaa gct gtc      144
Glu His Asp Val Gln Ile Glu Gly Val Arg Val Gly Leu Glu Ala Val
        35                  40                  45

gag ctg gat gat gga gca gct gtc ccc aag gag ttt gcc aat ccc act      192
Glu Leu Asp Asp Gly Ala Ala Val Pro Lys Glu Phe Ala Asn Pro Thr
    50                  55                  60

gat gac act ttc atg gtc gaa gat gcg gtg gaa gcc att ggg ttc gga      240
Asp Asp Thr Phe Met Val Glu Asp Ala Val Glu Ala Ile Gly Phe Gly
65                  70                  75                  80

aga ttc cag tgg aag ctc tct gtt ctc acc ggc ttg gct tgg atg gcg      288
Arg Phe Gln Trp Lys Leu Ser Val Leu Thr Gly Leu Ala Trp Met Ala
                85                  90                  95

gac gcc atg gag atg atg att ctg agc atc ctg gcg cct cag ctg cac      336
Asp Ala Met Glu Met Met Ile Leu Ser Ile Leu Ala Pro Gln Leu His
            100                 105                 110

tgc gag tgg cga ctc ccc agc tgg cag gtg gcg ctg ctg act tcg gtg      384
Cys Glu Trp Arg Leu Pro Ser Trp Gln Val Ala Leu Leu Thr Ser Val
        115                 120                 125

gtc ttc att ggt atg atg tcc agt tct acg ctc tgg gga aac atc tcg      432
Val Phe Ile Gly Met Met Ser Ser Ser Thr Leu Trp Gly Asn Ile Ser
    130                 135                 140

gat cag tat ggc agg aaa aca ggg ctg aag atc agt gtg ttc tgg acc      480
Asp Gln Tyr Gly Arg Lys Thr Gly Leu Lys Ile Ser Val Phe Trp Thr
145                 150                 155                 160

ctg tac tac ggc atc ctc agc gct ttc gcg cca gtg tat agc tgg atc      528
Leu Tyr Tyr Gly Ile Leu Ser Ala Phe Ala Pro Val Tyr Ser Trp Ile
                165                 170                 175

ctg gtg ctc cga ggc ctc gtg ggc ttt ggg att gga ggg gtg cct cag      576
Leu Val Leu Arg Gly Leu Val Gly Phe Gly Ile Gly Gly Val Pro Gln
            180                 185                 190

tcg gtg acc ctg tac gcc gag ttc ctc ccc atg aag gcc aga gcc aag      624
Ser Val Thr Leu Tyr Ala Glu Phe Leu Pro Met Lys Ala Arg Ala Lys
        195                 200                 205

tgc att ttg ctg att gag gtt ttc tgg gcc atc ggg acc gtg ttc gag      672
```

```
Cys Ile Leu Leu Ile Glu Val Phe Trp Ala Ile Gly Thr Val Phe Glu
    210                 215                 220

gtg ctt ctg gct gtg ttt gtg atg ccc agc ctg ggc tgg cgc tgg ctg      720
Val Leu Leu Ala Val Phe Val Met Pro Ser Leu Gly Trp Arg Trp Leu
225                 230                 235                 240

ctg ctg ctg tcg gcc gct cca cta ctt gtc ttt gct gtt ctg tgt ttc      768
Leu Leu Leu Ser Ala Ala Pro Leu Leu Val Phe Ala Val Leu Cys Phe
                245                 250                 255

tgg ctg cca gag agt gct agg tac gat gtg ctg tct ggg aac cag gaa      816
Trp Leu Pro Glu Ser Ala Arg Tyr Asp Val Leu Ser Gly Asn Gln Glu
            260                 265                 270

aag gcg att gct acc tta aag cgg atc gca aca gaa aat gga gcc ccc      864
Lys Ala Ile Ala Thr Leu Lys Arg Ile Ala Thr Glu Asn Gly Ala Pro
        275                 280                 285

atg cct ctg ggg aag ctc atc atc tcc aga cag gaa gac cga ggc aaa      912
Met Pro Leu Gly Lys Leu Ile Ile Ser Arg Gln Glu Asp Arg Gly Lys
    290                 295                 300

atg agg gac ctt ttc aca ccc cac ttt cgt tgg aca act ctg ctg ctg      960
Met Arg Asp Leu Phe Thr Pro His Phe Arg Trp Thr Thr Leu Leu Leu
305                 310                 315                 320

tgg ttt ata tgg ttc tcc aat gcc ttc tct tat tac ggc ttg gtt ctg     1008
Trp Phe Ile Trp Phe Ser Asn Ala Phe Ser Tyr Tyr Gly Leu Val Leu
                325                 330                 335

ctc acc aca gaa ctc ttc cag gcc gga gat gtt tgc agc atc tcc agc     1056
Leu Thr Thr Glu Leu Phe Gln Ala Gly Asp Val Cys Ser Ile Ser Ser
            340                 345                 350

cgg aag aag gcg gtg gaa gcc aaa tgc agc ctg gct tgt gag tac ctc     1104
Arg Lys Lys Ala Val Glu Ala Lys Cys Ser Leu Ala Cys Glu Tyr Leu
        355                 360                 365

agc aaa gag gat tac atg gac ctg ctg tgg acc acc ctg tct gag ttc     1152
Ser Lys Glu Asp Tyr Met Asp Leu Leu Trp Thr Thr Leu Ser Glu Phe
    370                 375                 380

cca ggt gtc ctt gtg act ctg tgg gtc atc gac cgc ctg ggc cgc aag     1200
Pro Gly Val Leu Val Thr Leu Trp Val Ile Asp Arg Leu Gly Arg Lys
385                 390                 395                 400

aag acc atg gct ctg tgt ttc gtc atc ttt tcc ctc tgc agc ctc ctg     1248
Lys Thr Met Ala Leu Cys Phe Val Ile Phe Ser Leu Cys Ser Leu Leu
                405                 410                 415

ctg ttc atc tgc att gga aga aat gtg cta acc ctc tta ctg ttc att     1296
Leu Phe Ile Cys Ile Gly Arg Asn Val Leu Thr Leu Leu Leu Phe Ile
            420                 425                 430

gca aga gcg ttt att tct gga ggc ttc caa gca gcc tac gtt tac acg     1344
Ala Arg Ala Phe Ile Ser Gly Gly Phe Gln Ala Ala Tyr Val Tyr Thr
        435                 440                 445

cct gag gtg tat cca acg gcg acg agg gcg ctg ggc ctg ggc acc tgc     1392
Pro Glu Val Tyr Pro Thr Ala Thr Arg Ala Leu Gly Leu Gly Thr Cys
```

```
    450                 455                 460

agc ggc atg gcg aga gtg ggc gcg ctc atc act cca ttc ata gct cag     1440
Ser Gly Met Ala Arg Val Gly Ala Leu Ile Thr Pro Phe Ile Ala Gln
465                 470                 475                 480

gtg atg ctg gaa tct tcc gtg tac ctg acc ctg gcc gtc tac agt ggc     1488
Val Met Leu Glu Ser Ser Val Tyr Leu Thr Leu Ala Val Tyr Ser Gly
                485                 490                 495

tgc tgc ctc ctt gct gcc ttg gcc tcc tgc ttt ctg ccc atc gag acc     1536
Cys Cys Leu Leu Ala Ala Leu Ala Ser Cys Phe Leu Pro Ile Glu Thr
            500                 505                 510

aaa ggc cga gca ctg cag gag tcc agc cac cgg gag tgg ggc cag gag     1584
Lys Gly Arg Ala Leu Gln Glu Ser Ser His Arg Glu Trp Gly Gln Glu
        515                 520                 525

atg gtt ggc cga ggg aca aac agc aca ggc gtc ccc agg tcg aac tct     1632
Met Val Gly Arg Gly Thr Asn Ser Thr Gly Val Pro Arg Ser Asn Ser
    530                 535                 540

ggc tct cag gag tag                                                1647
Gly Ser Gln Glu
545
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<210> 16
<211> 548
<212> PRT
<213> Rattus norvegicus

<400> 16

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Met Glu Glu Asp Leu Phe Gln Leu Arg Gln Leu Pro Val Val Lys Phe
1               5                   10                  15

Arg Arg Thr Gly Glu Ser Ala Arg Ser Glu Asp Asp Ala Ala Ser Gly
            20                  25                  30

Glu His Asp Val Gln Ile Glu Gly Val Arg Val Gly Leu Glu Ala Val
        35                  40                  45

Glu Leu Asp Asp Gly Ala Ala Val Pro Lys Glu Phe Ala Asn Pro Thr
    50                  55                  60

Asp Asp Thr Phe Met Val Glu Asp Ala Val Glu Ala Ile Gly Phe Gly
65                  70                  75                  80

Arg Phe Gln Trp Lys Leu Ser Val Leu Thr Gly Leu Ala Trp Met Ala
                85                  90                  95

Asp Ala Met Glu Met Met Ile Leu Ser Ile Leu Ala Pro Gln Leu His
            100                 105                 110

Cys Glu Trp Arg Leu Pro Ser Trp Gln Val Ala Leu Leu Thr Ser Val
        115                 120                 125

Val Phe Ile Gly Met Met Ser Ser Ser Thr Leu Trp Gly Asn Ile Ser
    130                 135                 140

Asp Gln Tyr Gly Arg Lys Thr Gly Leu Lys Ile Ser Val Phe Trp Thr
```

```
145                 150                 155                 160

Leu Tyr Tyr Gly Ile Leu Ser Ala Phe Ala Pro Val Tyr Ser Trp Ile
                165                 170                 175

Leu Val Leu Arg Gly Leu Val Gly Phe Gly Ile Gly Gly Val Pro Gln
            180                 185                 190

Ser Val Thr Leu Tyr Ala Glu Phe Leu Pro Met Lys Ala Arg Ala Lys
        195                 200                 205

Cys Ile Leu Leu Ile Glu Val Phe Trp Ala Ile Gly Thr Val Phe Glu
    210                 215                 220

Val Leu Leu Ala Val Phe Val Met Pro Ser Leu Gly Trp Arg Trp Leu
225                 230                 235                 240

Leu Leu Leu Ser Ala Ala Pro Leu Leu Val Phe Ala Val Leu Cys Phe
                245                 250                 255

Trp Leu Pro Glu Ser Ala Arg Tyr Asp Val Leu Ser Gly Asn Gln Glu
            260                 265                 270

Lys Ala Ile Ala Thr Leu Lys Arg Ile Ala Thr Glu Asn Gly Ala Pro
        275                 280                 285

Met Pro Leu Gly Lys Leu Ile Ile Ser Arg Gln Glu Asp Arg Gly Lys
    290                 295                 300

Met Arg Asp Leu Phe Thr Pro His Phe Arg Trp Thr Thr Leu Leu Leu
305                 310                 315                 320

Trp Phe Ile Trp Phe Ser Asn Ala Phe Ser Tyr Tyr Gly Leu Val Leu
                325                 330                 335

Leu Thr Thr Glu Leu Phe Gln Ala Gly Asp Val Cys Ser Ile Ser Ser
            340                 345                 350

Arg Lys Lys Ala Val Glu Ala Lys Cys Ser Leu Ala Cys Glu Tyr Leu
        355                 360                 365

Ser Lys Glu Asp Tyr Met Asp Leu Leu Trp Thr Thr Leu Ser Glu Phe
    370                 375                 380

Pro Gly Val Leu Val Thr Leu Trp Val Ile Asp Arg Leu Gly Arg Lys
385                 390                 395                 400

Lys Thr Met Ala Leu Cys Phe Val Ile Phe Ser Leu Cys Ser Leu Leu
                405                 410                 415

Leu Phe Ile Cys Ile Gly Arg Asn Val Leu Thr Leu Leu Leu Phe Ile
            420                 425                 430

Ala Arg Ala Phe Ile Ser Gly Gly Phe Gln Ala Ala Tyr Val Tyr Thr
        435                 440                 445

Pro Glu Val Tyr Pro Thr Ala Thr Arg Ala Leu Gly Leu Gly Thr Cys
    450                 455                 460

Ser Gly Met Ala Arg Val Gly Ala Leu Ile Thr Pro Phe Ile Ala Gln
465                 470                 475                 480
```

```
Val Met Leu Glu Ser Ser Val Tyr Leu Thr Leu Ala Val Tyr Ser Gly
                485                 490                 495

Cys Cys Leu Leu Ala Ala Leu Ala Ser Cys Phe Leu Pro Ile Glu Thr
            500                 505                 510

Lys Gly Arg Ala Leu Gln Glu Ser Ser His Arg Glu Trp Gly Gln Glu
        515                 520                 525

Met Val Gly Arg Gly Thr Asn Ser Thr Gly Val Pro Arg Ser Asn Ser
    530                 535                 540

Gly Ser Gln Glu
545
```